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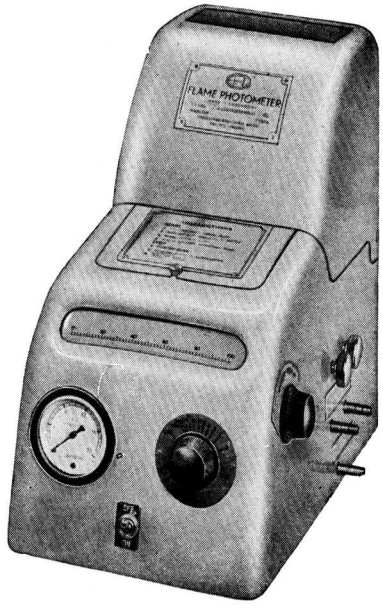

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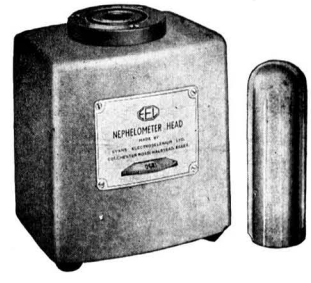

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CONTAMINATION PROBLEMS IN SOIL AND PLANT ANALYSIS*

By R. L. MITCHELL

The problems arising from contamination in soil and plant analysis are important chiefly in trace element work and originate either during the sampling and preparative stages or in the laboratory. With soils the chief danger occurs during transport in unsuitable containers or during drying and sieving. Plants are liable to be contaminated by soil, and means of assessing its severity and its possible effect on analytical results by determination of the apparent Ti content of the plant material are described. Contamination arising in the laboratory can be minimised by stringent precautions at all stages of the work, and by the use of carefully selected and, if necessary, purified reagents.

Introduction

The aim of any analytical procedure is to obtain a determination which gives a true assessment of the required constituent as it was at the time of sampling. No matter how exact or how elegant the analytical technique may be, little purpose is served if the amount of constituent precisely determined in the laboratory is not the amount present at the time of sampling.

The problems of analytical contamination can be divided into two aspects: (1) physical or surface contamination of samples which arise before, during or after sampling but before actual analytical processes begin, and (2) chemical or laboratory contamination which can occur in the course of the determination. Many plant materials are subject to surface contamination because of their format and habitat, and this is important since they may contain much smaller amounts of many constituents than do possible contaminants such as atmospheric dust, soil material or the many other extraneous substances which can cause trouble.

Contamination problems at the sampling stage are particularly liable to arise because of ignorance, inexperience or carelessness, and the extent of possible danger depends on the analytical information which is subsequently to be derived from the sample. If trace elements are to be determined, then stringent precautions must be taken from the outset. Much valuable laboratory time is expended on samples which would be discarded if the analyst knew how they had been treated before reaching the laboratory. Consultation regarding analytical requirements should take place before the sample is taken, and details of any precautions necessary should be passed in full to the actual sampler, both at the commencement of a series of investigations and at any stage at which a change in personnel occurs. In general, it appears advisable to make the requirements for trace analysis obligatory for all samples, as otherwise the relaxations permitted when sampling for limited analysis for major constituents will tend to be adopted when they are not permissible. Moreover, determinations of trace elements are often required on diagnostic samples initially taken for analysis for major elements, should this not give a conclusive result.

In the following discussion, certain of the factors are of major importance, others may be considered to be trivial and given undue prominence. Nevertheless, even the most trivial can on occasion result in an entirely erroneous finding.

Sampling of soils

There are not many possibilities of serious contamination of soils at the actual sampling stage. The soil auger or soil borer must, of course, be clean and free from oil or grease, and be made of mild steel, not stainless steel, brass or galvanised iron if there is any chance of trace-element determinations subsequently being carried out. The problem of drawing a representative sample is scarcely relevant, although it may be necessary to point out that care should be taken to ensure that the sample represents, in the correct proportion, material from all parts of the required layer. Sampling points will be chosen to avoid obvious accumulations of droppings or fertilisers, or areas showing the effects thereof, and enough sub-samples taken to ensure

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

an adequately representative sample. The greatest danger arises from extraneous sources, among which may be mentioned fertilisers and other agricultural materials commonly found in the farmyard and which may inadvertently contaminate the soil sample. Soil samples should not be mixed in farm buckets, which are a very probable source of contamination. They should be packed for transport in clean, dust-proof containers, such as lined paper or plastic bags, and properly labelled. The most drastic instance of contamination arises when the sample contains none of the material which it is desired to analyse! This occurs when samples are wrongly identified.

The safest packaging material is probably a tough polythene bag. Polythene is relatively free from major or trace contaminants, is non-porous and easily cleaned, although the bags are rather easily damaged. The common alternative is a paper bag lined with greaseproof paper. An unlined bag readily disintegrates in contact with moist soil and so introduces contamination. Paper containers should not be used for samples which are sufficiently moist to lead either to extraction of impurities from the paper by dissolution, or to incorporation of pieces of paper by disintegration. The possible metallic constituents of paper bags are very variable. Samples which have been examined in this laboratory have shown 1–10% of ash containing, in addition to the normal major constituents of plant material, up to 0·2% of such elements as Cr, Cu, Mn, Ni, Pb and Zn, together with up to 100 p.p.m. of Ag, Be, Co, Ga, Mo, Sn, Sr, V, Y and Zr. One bag with a bitumen-impregnated layer contained over 1% of V in the ash. There appeared from the somewhat limited number of samples examined to be little difference, from the metallic content of the ash, between brown paper and white lining, although the latter is desirable because it is less affected by mechanical abrasion and moisture. Winsor[1] has reported B contamination of soil samples collected in paper bags, of the order of an 100% increase in the amount found.

Metallic containers should be avoided, as contamination during transport is readily introduced. In one test to ascertain the possible magnitude of this effect, a sample of quartz sand shaken in a tin box fitted with a galvanised iron base and a copper lid picked up Cu 11 p.p.m., Sn 2 p.p.m. and Zn 15 p.p.m. soluble in dilute acetic acid. These amounts are several times the extractable contents found in normal soils.

Soils are best dried just above room temperature, spread out on clean polythene or greaseproof paper on trays of wood or aluminium. Forced ventilation should not be powerful enough to cause disturbance of fine clay particles. Dry soils are passed through a 2-mm. screen after rolling with a hardwood roller to break down lumps. The best sieve is probably of steel or aluminium. Galvanised sieves or sieve boxes should be avoided, as should vigorous shaking in a closed metal sieving chamber. Suction extraction is desirable to protect the operator from dust, but this should not be powerful enough to remove fast-falling particles, or to disturb the finest material from soil lying in the vicinity of the extract vent. Various types of mechanical soil-sieving machines are coming into use for routine advisory analysis. These introduce many possibilities of trace-element contamination and should not be used without thorough investigation.

Dry soils are conveniently stored in paper bags or waxed paper cartons. Sub-samples should not be removed by metal spatula or spoon, but the required weight obtained by quartering the whole sample, in view of the tendency of soils to undergo some size fractionation.

Sampling of plant materials

Whilst it is relatively simple to take an uncontaminated soil sample, it is much more difficult to obtain a good plant sample in the field. There is always the possibility of surface contamination by fine dust from soil or other sources which contain very much higher contents of many elements than do the plants themselves.

The most readily available contaminant is the soil on which the plant is growing, and the extent of possible contamination depends largely on the form of the plant being investigated. The leaves of trees and high-growing plants are less liable to suffer soil contamination than are pasture herbages and vegetables. Foliage of vegetables such as potatoes which are covered by fine hairs are particularly susceptible, while pasture herbage can be affected by being brought into mechanical contact with the soil by trampling by grazing animals. Weather conditions

control soil contamination of plants to a marked degree. While heavy rainstorms may help to wash tall plants, they contaminate plants of low format by splashing, and samples should be taken neither following heavy precipitation, nor after a prolonged dry spell when they may be particularly dusty.

Soil particles tend to become lodged in parts of plants such as leaf nodes as they emerge from the ground, and this may introduce quite large sand grains compared with the fine clay which becomes associated with leaf surface. A form of over-all surface contamination of rather more unpredictable nature occurs in the neighbourhood of sources of fine dusts, such as busy roads, quarries, and industrial establishments which emit smoke or fumes. In such instances, the surface contamination may be an important part of the sample and the analysis may be carried out to establish its nature.

The significance of any given degree of contamination depends on the element to be determined and its relative amounts in the uncontaminated sample and in the contaminant. The permissible limit of contamination for any element depends on the ratio of its contents in the sample and in the contaminant, not on its absolute amount in either. It is possible to draw up a rather empirical table showing average contents in plant material and in the finer material of soils. Because of the variations between plants and between soils, Table I is necessarily somewhat approximate. As there is, for many elements, at least a tendency for soils with high contents to carry plants with correspondingly high contents, the assessment may not be so arbitrary as may at first sight appear. It cannot be employed without reserve for soils with abnormally high contents of certain trace elements, such as those of ultrabasic igneous origin, but such information should be available to the analyst. If in the element being determined an increase in content resulting from contamination of 5% of the amount present be acceptable, and this is a very conservative figure better than the normal analytical accuracy, then for an element with a soil/plant ratio of 10 the contaminating soil material must be present at a content of 0·5% in the dry plant material before difficulties arise. Even in unfavourable conditions this cleanliness should be achieved. It is only for elements with ratios of above 100 that due regard must be paid to the possibility of soil contamination in considering the accuracy of analytical results. It is particularly interesting to note that, of the accepted essential plant nutrients, only the Fe determination is readily affected by soil contamination. From Table I it can be seen that the possibility of soil contamination affecting determinations of P, K, B, Mo, Ca, Mn, Zn or Cu can generally be disregarded. The position is much more serious as far as Co is concerned. Only by herbage analysis can the animal intake be assessed, and a pasture intensively grazed by sheep or cattle is very liable to be contaminated with soil. The best way to avoid this is to restrict access to small areas by fencing—not by covers of wire-netting which may increase the Zn content abnormally, and possibly give rise to misleading conclusions. The enclosed herbage should be regularly cut to simulate grazing, otherwise the herbage sampled will not represent that eaten by the animals.

Table I

Estimated average total contents of the constituents of soil fine material and plant dry matter (p.p.m.) and the soil/plant ratio

	Soil	Plant	Ratio		Soil	Plant	Ratio
P	2000	4000	0·5	Mn	2000	100	20
K	20,000	20,000	1·0	Na	20,000	1000	20
B	10	10	1·0	Pb	20	1	20
Mo	1	1	1·0	Ni	50	1	50
Ca	30,000	20,000	1·5	Co	20	0·1	200
Zn	100	30	3·3	Fe	50,000	100	500
Cu	50	10	5·0	Cr	200	0·1	2000
Sr	300	30	10	V	200	0·1	2000
Mg	20,000	2000	10	Ti	10,000	1	10,000

It is possible to assess the extent of soil contamination of a pasture herbage by considering the apparent content in the herbage of elements with high soil/plant ratios.[2] The most favourable element for this purpose is Ti with a ratio of around 10,000 : 1. This test is 20 times as

sensitive as that using Fe or about 10 times that with Al, which has also been suggested as an indicator of soil contamination.[3] The Ti contents found for clean pasture herbages sampled during the growing season normally fall into the range 0·5–2 p.p.m. Except for some plants, such as heather or certain trees, values above 5–10 p.p.m. are not found in apparently clean samples, and even the contents around 1 p.p.m. may be partly contamination. As a ten-fold rise in Ti from 1 to 10 p.p.m. would result from soil contamination of the order of 0·1%, and would involve an increase of only 20% from 0·1 to 0·12 p.p.m. in the Co content of pasture herbage on a normal soil and probably less on a Co-deficient soil, a plant with a Ti content of this order can be taken as acceptable, but higher contents render Co determinations suspect. The previously mentioned level of 0·5% soil contamination is readily detected. This test is particularly useful when spectrographic methods of analysis are employed, as a Ti determination may involve little extra work.

A pasture herbage sample should be cut, not plucked, as this inevitably results in some roots being included, so introducing soil material. Steel sheep-shears have been found satisfactory. If both plant and soil samples have to be taken, the former should always be taken first, in order to avoid handling plants with soiled hands.

The sampling of other types of plant material is generally more straightforward. The required portion should be cut or picked and placed immediately into appropriately labelled containers. Polythene or lined paper bags are again the most suitable for this purpose. As Steyn[4] points out, the keeping quality of fresh plant material in polythene is poor. Cloth bags should not be used, as they are too porous and readily allow contamination during transport. They are particularly objectionable when plants and soils are parcelled together. When a whole plant with roots is to be examined, special care is needed to ensure that soil from the roots does not reach the leaves.

If plant samples cannot be air-dried immediately, or if some preliminary treatment, such as separation of mixed herbage into individual species, be required, samples should be kept in a refrigerator at a temperature near freezing point.

Some distinction must be made between the leaves of graminae and conifer needles on the one hand and the larger leaves of vegetables and deciduous trees on the other, in considering possible measures to remove contamination. It is seldom practicable to attempt to clean any samples in the former category after sampling. This applies particularly to pasture herbage. The nature of the material and the amount generally required (sufficient to give 50–100 g. of dry matter) would render the task exceedingly tedious. There is no information regarding the possible extraction of trace constituents from material of this type by any of the various cleaning solutions which have been proposed.

A considerable amount of work has been carried out on the cleaning of contaminated tree leaves. This has related chiefly to the determination of iron. Mason[5] has shown that treatment of apple leaves with 0·3N-HCl caused an immediate reduction in Fe content from 170 to 110 p.p.m. in the dry matter by removal of surface contamination, followed by a very slight fall over a period of 100 min. due to leaching. With K the leaching effect was considerably greater, almost half of the original 1% K being lost, although there was no great initial fall during the first few minutes. Mason recommends immersion of individual leaves in 0·2% Teepol (a sodium alkyl sulphate detergent) for 10 sec. during which the leaf is scrubbed by a soft nylon toothbrush, followed by rinsing in distilled water. Nicholas *et al.*[6] and Steyn[4] also recommend washing with Teepol. The former checked the efficiency of the washing process with radioactive Fe and found no removal of internal Fe. The fact that Steyn found some 20% reduction in Cu after washing suggests, in view of the figures quoted in Table I, that soil material was not the active contaminant in the samples he examined, or that leaching was occurring. This cleaning technique is obviously not very practicable when many samples, each of up to 100 g., are required, as is the case in certain types of comprehensive investigations of trace elements. Mason points out that leaching is more rapid from dead than from living leaves, so that samples should be cleaned as soon as possible after collection. No information appears to be available on the possible uptake of trace elements from cleaning solutions.

Plants grown in normal greenhouses cannot be considered to be free from surface contamination. Samples of greenhouse dusts analysed at the Macaulay Institute showed the follow-

ing approximate contents of trace elements in p.p.m. as well as major constituents Si, Al, Ca, Na and K in amounts similar to those in soils:

Ag	1	La	50	Pb	5000	Ti	4000
Ba	1500	Li	40	Rb	100	V	150
Co	25	Mn	600	Sb	400	Y	70
Cr	150	Mo	10	Sn	100	Zn	1200
Cu	200	Ni	100	Sr	700	Zr	700
Ga	10						

The presence of substantial amounts of such elements as Pb, Sb, Ti and Zn points to contamination from paint, which may also be the source of part of the Ba, Co, Mn, Sn and Zr in the dust. The effects of this dust would, for several elements, notably Cu, Pb, Sb, Sn and Zn, be much more serious than an ordinary soil dust. Similar materials can be encountered in the open air in the neighbourhood of industrial establishments, and each occurrence must be considered individually.

Drying of plant materials is best carried out in a ventilated electric oven, preferably aluminium-lined. Various workers choose temperatures from 65° (Steyn[4]) to 105° or even higher, although 80° is commonly chosen as the most suitable temperature.

A number of workers including Hood,[7] Lykken[8] and their co-workers have examined the possibilities of contamination during grinding. With a good mild-steel hammer-mill there is obviously a chance of Fe contamination, but in our experience, with material of the texture of pasture herbage, this amounts to no more than 1 or 2 p.p.m. in a total of 50–100 p.p.m. with a Christy & Norris mill. When mills are employed for trace analysis work all scoops, trays and screws of copper or brass should be replaced by aluminium, steel or plastic. The material used for bearings should also be checked. The new molybdenum sulphide lubricants may be a danger if their use in laboratory equipment develops.

Where only small samples are required, agate ball-mills or mortars can be employed (Steyn[4]), but they are not very practicable with samples of the order of 100 g. Kretschmer & Randolph[9] have described a nylon slip roll pulveriser which eliminated contamination, while Palm & Beckwith[10] have modified the Christy & Norris mill to avoid iron contamination. In general, if reasonable precautions are taken, contamination by grinding is probably serious only for Fe in detailed studies of that element, and can be ignored in most trace-element work, although brass, stainless steel or plated mill parts must always be suspect.

Laboratory precautions

There are so many possible sources of trouble when determinations of trace elements are attempted in an ordinary chemical laboratory that it is not feasible to attempt to detail them. With the introduction of modern methods for major constituents operating at levels much lower than those applicable to the classical chemical techniques, the same precautions may be required even for such elements as calcium and magnesium. For the determination of the major constituents of soils and plants by chemical methods, the normal standards of laboratory cleanliness applicable to all analytical work are adequate.

The requirements for trace elements can best be illustrated by outlining some points in the design of the new spectrochemical laboratory of the Macaulay Institute now being built. The construction is conventional with plastered walls, linoleum-covered heated floor and teak benches with wooden cupboard and drawer units. The walls will be painted with an amide-cured epoxide resin paint, with the restriction that titanium be the only metallic pigment. Lead, zinc and other biologically important metals are to be excluded. Metallic driers such as cobalt have also been eliminated. All exposed metal-work has been avoided as far as practicable. Electric switches and sockets are plastic covered, as are the fluorescent electric light fittings, under-bench pyrotenax cables and copper hot water pipes. Other metal pipes, including those for gas and compressed air, are to be protected with a silicone-based aluminium paint, which has been found to be very useful for general laboratory use. Cold water supplies, sink fittings and waste pipes will be of polythene. Plastic-wheeled remote-control taps will control all water and gas services, so avoiding handling of metal as far as possible, the outlets being PVC covered. The fume cupboards are to be entirely lined with PVC, with hot plates and water baths mounted flush in

a hard asbestos false floor. Corrosion-resistant water baths are desirable, and polypropylene would appear to be the most suitable material for the bath itself. For a number of years aluminium–5% magnesium alloy hot-plates have been employed in place of the usual readily-corroded iron hot-plates. They have given good service in an atmosphere of acetic acid, hydrochloric acid and nitric acid. The presence of Mg is undesirable, but more acceptable than Cu, a major constituent of dural-type alloys. Copper contamination also arises from the normal bunsen burner: it has been found impracticable to ash small amounts of organic materials in a crucible over a normal burner without increasing its Cu content appreciably. The remedy is to fit silica burner tubes or to use electric burners with silica sheaths over the heater elements. Silica-lined muffle furnaces and aluminium-lined ovens are the most suitable available at present. Equipment using nickel, zinc or cadmium plating should generally be avoided in trace-element work.

The equipment chosen for the various analytical processes will depend on the actual determination involved. For much trace-element work, other than B, glass is reasonably safe, but porcelain should not be used if damage to the glaze is possible. Ashing is best carried out in platinum, which on a long-term basis is reasonably economical, but which can be contaminated with Ni or Pb from biological materials if not properly used. Silica is acceptable for many purposes, but can introduce elements such as Li which are used as fluxes. The best of the plastics materials is high-pressure polythene: the newer types of polythene, made by the Ziegler or other processes, are liable to contain appreciable amounts of such catalysts as Fe, Al, Ti, V or Mo. Quite large amounts of Cu, Pb, Sn, Cd, Ti and other elements can occur in polyvinyl chloride, polymethyl methacrylate and polystyrene. Whether or not these are extracted depends on the treatment they receive. Surface contamination of plastic mouldings with Zn can arise from lubricants such as zinc stearate, and all such materials must be thoroughly cleaned before use, as this compound is readily soluble. A discussion of the qualities of various plastic materials is given by Thiers,[11] who presents figures for loss or gain at the parts per thousand million level of various elements in glass and plastic containers after 16 months' storage.

Rubber is quite liable to introduce contamination. On replacing the rubber stoppers of soil shaking bottles by polythene caps, the apparent amount of Zn extracted from soils by dilute acetic acid fell from around 15 p.p.m. to below 5 p.p.m. Rubber stoppers, rubber tubing and rubber ' policemen ' should therefore be used with discretion in trace-element work. Polythene rods tapered at one end make very effective ' policemen ' for removing precipitates from glassware.

Laboratory glassware and other equipment should be retained specifically for trace-element work, and precautions taken to ensure that determinations such as those involving the weighing of dry precipitates of phosphomolybdate or cobaltinitrite are not carried out in proximity to the trace-element laboratory. As far as possible, dry soils should not be handled in the laboratories, but should be quartered and weighed out in a room provided with dust extraction, adjoining the soil store. Separate rooms should be available for plant preparation if work involves both soils and plants.

In all work involving determinations at the μg.-level—a common magnitude for trace elements or even elements such as magnesium or the alkali metals by spectrochemical methods—stringent general cleanliness must be maintained in the laboratory. All cleaning should be by vacuum cleaners and any necessary mopping up done with moist cloths to avoid raising dust, and waste buckets taken outside the laboratory before emptying. Laboratory personnel must be instructed regarding possible sources of contamination, such as talcum powder, face powder or medical preparations such as boric ointment or zinc plaster. If smoking be permitted rigid control is essential to avoid dispersal of ash.

Purification of laboratory reagents

Tap water is seldom suitable for use in analytical determinations, but care must be taken to ensure that any process of purification does not in fact introduce trace elements when eliminating the major impurities. In Aberdeen tap water, a relatively pure soft water, the contents of trace elements found in one series of analyses were, in μg./l. (parts per 10^9), Cu 17, Zn 26, Ni 0·5, Co <0·07, Mo 0·12, Pb <2, Sn <1, Ti <2, V 0·2 and Cr 0·4. Contents as high as

Cu 300, Zn 2000, Ni 30, Co 10 and Pb 80 (all μg./l.) have been found in waters from other sources, probably arising either from contamination by water-pipes or from local geological factors. From such figures it can readily be deduced that unless the actual trace-element content of a tap water is precisely known it is not permissible to employ it for such purposes as washing plant material prior to analysis. When present at the 100 μg./l. or 0·1 p.p.m. level in wash water, appreciable amounts of Cu might be absorbed by plant material itself containing only 1–2 p.p.m. of Cu in the dry matter, corresponding to an amount in the fresh material of nearly the same order as in the wash water.

The standard method of purification is distillation in glass equipment, metal stills other than platinum being unsuitable for trace-element work. For many purposes ion-exchange resin purification is equally acceptable, particularly with soft waters, and is much more conveniently effected. It has been found that the simple Ecam all-glass still (no longer in production) reduces the trace-element content of our laboratory water after a single distillation to Ni <0·3, Mo <0·07, Zn 9 and Cu 0·37 μg./l. A small mixed-bed resin exchanger, the Elgastat B102, has repeatedly given results of a similar order for most elements, the Zn contents being somewhat better at about 0·5 μg./l., although the Cu contents, which commence at around 0·3 μg./l. with fresh resin, tend to increase to about 20 μg./l., the level in the original water, as the resin approaches the end of its useful life, which is about 400 litres of Aberdeen tap water per exchangeable cartridge carrying some 500 g. of resin. An appreciable increase in conductivity and in Na content is apparent at the same time.

Results quoted by Thiers[11] indicate findings of a similar order for single distillation or simple ion-exchange purification. Much lower figures were reported with long exchange columns or with repeated treatment. For normal trace analysis of soil and plant samples, the levels obtained by the simpler means detailed above should generally be adequate. There is little point in preparing acceptable water then allowing it to become contaminated. The dispenser should probably be of polythene, with polythene tubing and short lengths of plasticised PVC. If a tap or clip be fitted to the distilled water reservoir it should be basically non-metallic, not nickel-plated as are many spring clips used for such purposes.

Chemical reagents provide the most diverse sources of contamination in analytical processes. If the total amount of any element introduced with the reagents and from other reproducible sources is such that the true amount in the sample can be accurately determined by making a blank correction, then this course can be followed, but if the amount introduced is considerably greater than the amount in the sample, or is not reproducible, then an accurate determination is impossible and steps must be taken to purify the reagents. The limiting levels depend on the relative contents, on the degree of accuracy desired and on the precision of the assessment.

It is quite impossible to discuss all possible types of reagent contamination or the methods of purification. There are certain common sources which must be continuously guarded against; others may be infrequent and quite unpredictable. The normal liquid reagents, such as acids and aqueous ammonia, are frequent sources of metallic contamination. Thus, analytical-reagent grade nitric acid frequently contains Ni, and aqueous ammonia Mo, in unacceptable amounts. The common acids can generally be obtained in a sufficiently pure state by distillation at the constant-boiling concentration, while ammonia of adequate strength is most economically prepared by bubbling ammonia gas from a cylinder into suitably purified water.

It will sometimes be found possible to obtain a reagent sufficiently free from the required elements among commercial grade chemicals which have not been subjected to contamination by traces of metals during purification processes such as crystallisation in stainless steel vessels. (V. M. Goldschmidt always insisted that the purest sodium carbonate from the trace-element aspect came from the stock of washing soda in the village shop!). We have found ordinary grade ferric oxide less contaminated with Co and Ni than a German finest analytical-reagent grade, although the former did contain a large amount of silica. Trace metals can be removed from reagents such as sodium carbonate by extraction with organic reagents such as dithizone after dissolution in water. Materials such as alumina required in spectrochemical work can be prepared by removal of iron by ether extraction in acid solution followed by precipitation as hydroxide or quinolate. Selection of the appropriate stage of fractional precipitation or crystallisation will often enable a product free from the unwanted constituent to be obtained.

A number of the techniques adopted at the Macaulay Institute have been described elsewhere.[12, 13] Often an appropriate starting material free from some less readily eliminated contaminant has to be chosen. Thus, when preparing pure Al_2O_3, an Al salt free from Cr is the preferred starting material. Little can be done, in our experience, with a pre-war sample of analytical-reagent grade sodium citrate containing over 10% of potassium, but the possibility must be kept in mind. Full records should be kept of batch numbers of all types of reagents (bought or laboratory-prepared) and filter papers, in order to trace the source of any discrepancies which may subsequently be discovered. In conclusion, although it is not completely relevant to the subject of this communication, attention must be called to the full discussion of contamination problems and purification processes in solution and pot culture work by Hewitt.[14]

Conclusion

In dealing with the problems which arise from contamination there is only one safe policy, to suspect every process to which the sample is subjected and to check that every reagent and piece of equipment is suitable for the purpose in view, before wasting time and materials on valueless analyses. Blank determinations can on occasion be much more useful when carried out prior to rather than concurrently with analyses, although they are desirable at that stage also.

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ANALYSIS OF THE STRUCTURAL CARBOHYDRATES OF HERBAGE*

By I. H. BATH

A method is proposed for the fractionation of the carbohydrates of plant material by successive extractions with ethanol–benzene, 95% ethanol and hot water followed by delignification and extraction of the isolated holocellulose with potassium hydroxide solution to yield hemicellulose and α-cellulose. The polysaccharides in the hot water extract, hemicellulose and α-cellulose are hydrolysed and the constituent monosaccharides separated by paper chromatography and determined spectrophotometrically.

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

Introduction

The carbohydrates in herbage are the most important sources of energy for ruminants. The laboratory methods by which the nutritive value of pasture, hay, dried grass and silage is assessed normally depend upon the division of the carbohydrates into two groups, crude fibre and nitrogen-free extractives. Many authors have drawn attention to the fact that this division is an arbitrary one[1–10] and some have suggested modifications or proposed other methods. Reliable procedures exist for the determination of the more soluble carbohydrates[11] but little attention has been given to the detailed composition of the structural carbohydrates which form the principal constituents of fresh and conserved herbage feedstuffs.

During the course of an investigation of the plant and animal factors affecting the nutritive value of the structural carbohydrates of herbage for the dairy cow[12] the scheme presented here for the fractionation and analysis of plant material was devised. A fuller account of the choice of experimental conditions, etc. is given by Bath.[12]

Experimental

Preparation of the sample

The more soluble portions of the cell wall are subject to rapid enzyme breakdown after the harvesting of a sample of fresh herbage and rapid inactivation of the enzyme systems in the living material is necessary. The method employed for the dehydration of herbage exerts a considerable influence on the ultimate constitution of the product[13, 14] and may affect the solubility of polysaccharides in the extraction solvents. Both drying by heat and by immersion in boiling alcohol have several disadvantages[15] and therefore a freeze-drying technique is preferable.

Fractionation of the plant material

(1) *Removal of lipids, pigments and soluble sugars.*—It is normally desirable to remove lipids and so-called extractives before proceeding with polysaccharide separation. This treatment not only eliminates them as a source of impurities but opens the tissue to penetration by hydrophilic solvents. The most widely used extractant is an azeotropic mixture of ethanol and benzene.

The removal of free sugars from the plant material without affecting the pectin fraction is the next stage. Extraction with an azeotropic mixture of ethanol and water for 2–4 h. removes all sucrose, glucose and fructose and no polysaccharides.[11, 16]

(2) *Extraction of fructosan and pectin.*—Fructosan may be extracted by shaking the residue from the ethanol extraction with cold water for four periods of 3 h. each[16] but considerable amounts of other polysaccharides may be simultaneously extracted.[17] As fructose may be readily separated from the sugars of the pectin fraction by paper chromatography, fructosan is more conveniently extracted with the pectin. The infinite variety of reagents used to extract pectin from cell-wall preparations results in very different amounts of carbohydrate material being removed. In view of the complex structure of the plant cell wall, the division between pectin and hemicellulose is necessarily an arbitrary one. As these two fractions probably differ most in the nature of the sugar residues present it would appear, therefore, more satisfactory to compare reagents for the extraction of pectin by qualitative examination of the sugars present in the hydrolysed extract rather than by the total amounts of carbohydrate removed.

The use of water as an extractant for pectin simplifies the succeeding hydrolysis and chromatography since no contamination of the pectin or residue takes place. Further, little, if any, degradation of the pectin should occur and a fairly sharp boundary between pectin and hemicellulose should still be attained. A comparison has been made between two widely used reagents (ammonium oxalate and oxalic acid) and water as extractants of the pectin fraction.

A sample of H1 ryegrass containing a small percentage of S100 clover was dried and extracted as in (1) above. Five-g. samples were extracted with 250 ml. of (*a*) 0·5% ammonium oxalate solution, (*b*) 0·5% oxalic acid solution, both for 12 h. at 80–85°. (*c*) A third portion was refluxed with 250 ml. of boiling water for 12 h. (pH of the resultant solution was 5·8 and

6·0 in duplicate procedures). (*d*) The residue from treatment (*c*) was refluxed with a further 250 ml. of boiling water for 12 h.

Residues obtained on filtration were washed with hot water and the filtrate plus washings concentrated *in vacuo*. Ammonium oxalate and oxalic acid were removed by de-ionisation. After hydrolysis in 3% HNO_3 solution the sugars present were separated by paper chromatography and the developed chromatogram sprayed with *o*-aminodiphenyl hydrogen oxalate reagent.[18] The relative densities of the carbohydrate spots determined visually are given in Table I.

Table I

Comparison of ammonium oxalate solution, oxalic acid solution and water as solvents for pectin: relative density of the spots of sugars identified on the chromatograms

Treatment	Arabinose	Fructose	Galactose	Glucose	Xylose
(*a*)	++	++	+	++	o
(*b*)	++	++	+	++	+
(*c*)	++	++	+	++	±
(*d*)	+	o	o	+	+

Spot intensity code

++ Maximum intensity, very distinct spot
\+ Less than maximum intensity, light coloured spot
± Very faint spot, at limit of detection
o No spot

Treatment (*a*) 0·5% ammonium oxalate solution, 12 h. at 80–85°
(*b*) 0·5% oxalic acid solution, 12 h. at 80–85°
(*c*) boiling water, 12 h.
(*d*) residue from (*c*) boiling water, 12 h.

The three procedures appeared to be equally suitable for the extraction of pectin from the cell-wall preparation. The xylose present in the hydrolysates probably originated in the hemicellulose part of the cell wall as it is not a sugar typical of pectin, and this is substantiated by the presence of more xylose in the second water extract than in the first. The amount of xylose-containing polysaccharide present in the first water extract was extremely small and at the limit of detection on the chromatogram. All the other sugars were obtained in approximately equal amounts by the three main extraction procedures. As ammonium oxalate and oxalic acid must be removed from extracts before chromatography, the use of boiling water is obviously far more convenient. Jermyn[19] found that extraction of pear pomace with boiling water for 12 h. was sufficient to effect complete removal of polygalacturonic acid. There was no further loss in weight of the material after this period, nor was superior extraction secured with ammonium oxalate reagent. A single 12-h. extraction with boiling water, therefore, is considered satisfactory.

(3) *Removal of lignin.*—The acid-chlorite delignification method introduced by Jayme[20] and modified by Wise *et al.*[21] and others is satisfactory. The extent of the carbohydrate losses during the delignification depends on the duration and number of treatments. Lignin is substantially removed from grasses by relatively short extractions with acid-chlorite and the holocellulose yield after 30 min. treatment is almost quantitative.[22]

(4) *Extraction of hemicellulose.*—Wise *et al.*[21] fractionated chlorite holocellulose by successive extraction with 5% and 24% KOH. Insolubility in the latter strength of alkali has, ever since the work of Cross & Bevan,[23] served as the definition of ' α-cellulose '. Wise *et al.*[21] suggested extraction at two concentrations as they found a larger proportion of uronic acid groups in hemicellulose extracted with 5% than with 24% KOH from the residue of the first extraction. The choice of alkali concentrations is an arbitrary one, however, as abrupt changes in composition of the fractions resulting from extractions with alkali of increasing strength do not occur.[21, 24, 25] Jermyn[19] considers a single extraction procedure with 24% potassium hydroxide satisfactory provided the mixture of sugars obtained in the hemicellulose hydrolysate can be readily separated by paper chromatography. In the present study paper chromatographic methods are used which separate all the sugars found in the hydrolysates of herbage hemicelluloses and, therefore, it is considered appropriate to extract the whole of the hemicellulose fraction by a single treatment with 24% KOH.

Hydrolysis of the polysaccharide fractions

(1) *Pectin and hemicellulose.*—HNO_3, 3% v/v, free from oxides of nitrogen and containing a little urea, is a satisfactory reagent for hydrolysis of hemicelluloses and material with high contents of uronic acids and pentoses.[26, 27] Quantitative yields were obtained[19] from pentosans in 3 h. at 100°, but uronic acids require a longer time and some loss of sugar occurs (e.g., galacturonic acid determined in pear cell wall pectin was 90% of that determined by total hydrolysis with galacturonidase[28]). Two samples are therefore hydrolysed, one for 3–4 h. for determination of sugars from a polysaccharide, and the other 12 h. for the uronic acids. In the present work, chromatographic examination of the 12-h. hydrolysate of the hemicellulose fraction of herbage material indicated the presence of uronic acid polymers of unknown composition, which were not obtained from the pectin fraction. These were probably aldobiouronic acid end products, containing xylose and uronic acid, which are known to be extremely resistant to acid hydrolysis[29] and do not liberate the uronic acid quantitatively. This portion of the hemicellulose fraction, therefore, has not been estimated.

(2) *α-Cellulose* is most conveniently hydrolysed by the routine method of Monier-Williams.[30]

Analysis of the hydrolysates for constituent monosaccharides

The mixture of carbohydrates obtained by hydrolysis of a polysaccharide fraction is most readily analysed by paper chromatography and the separated sugars and uronic acids determined by the simple ultra-violet spectrophotometric method of Bath.[31] It is essential to remove the HNO_3 before paper chromatography and for solutions containing sugars de-ionisation with Amberlite I.R.A.-400 resin (CO_3^{2-} form) is satisfactory (sugar yields 99·4–103%), but uronic acids are removed. Uronic acids may be separated by adsorption on a column of I.R.A.-400 or Dowex-1 resin in the acetate form, and elution with acetic acid.[32, 33] This procedure used batchwise is also suitable for removing nitric acid from uronic acid solutions. The recovery of galacturonic acid from 3% nitric acid solutions de-ionised with I.R.A.-400 (acetate) resin ranged from 97·9 to 101%.

Proposed scheme of analysis

Preparation of the sample

Fresh herbage is rapidly frozen to −15°, lightly packed into shallow trays and freeze-dried to reduce the moisture content of the material to less than 5%. The sample is milled to pass a 0·8-mm. sieve, care being taken to recover all the material, including that deposited within the mill itself which may have a different composition from that passing directly into the receiver.[34] The dry matter content of the herbage is determined by oven-drying at 100°.

Fractionation of the plant material

(1) *Removal of lipids, pigments and soluble sugars.*—Each sample (5 g.) is extracted for 4–6 h. with a mixture of ethanol and benzene (1 : 2 w/w) in a Soxhlet apparatus. The air-dry residue is similarly extracted with 95% w/w ethanol until no further pigment is removed (2–4 h.). Quadruplicate samples are extracted to obtain sufficient of the cell-wall preparation for the following analytical procedures to be carried out in duplicate.

(2) *Extraction of fructosan and pectin.*—Five g. of the cell-wall preparation are gently boiled with 250 ml. of water under reflux for 12 h., filtered while hot through a sintered-glass funnel (porosity 3) and washed with boiling water. The extract and washings are bulked together and concentrated *in vacuo* at 35° to a convenient volume for hydrolysis.

(3) *Removal of lignin.*—The method used is that of Wise *et al.*[21] One treatment with acid chlorite for 30 min. is normally sufficient, but hay and similar material may require a second treatment; the chlorite extract and washings must then be tested for carbohydrates and any polysaccharides extracted into solution during delignification isolated and estimated.[22]

(4) *Extraction of hemicellulose.*—The residue of holocellulose from (3) (1·5 g.) is weighed into a tared 250-ml. B.24 conical flask and 225 ml. of 24% w/v KOH solution added. Oxygen-free nitrogen is bubbled through the solution for 10 min. to remove the air and the flask then immediately sealed with a ground-glass stopper, kept in position with cellulose self-adhesive

tape. The flask is mechanically shaken for 110 min. The contents of the flask are filtered through a tared sintered-glass funnel into 100 ml. of acetic acid in the suction flask. The flask and stopper are washed with 25 ml. of 24% KOH. The residue is washed with water until free of alkali and then with 25 ml. of 10% acetic acid solution followed by water. The residue is α-cellulose.

The filtrate plus washings are adjusted to pH 4·0 with acetic acid and 3·5 volumes of ethanol are added. After keeping overnight the precipitated hemicellulose is collected on a sintered-glass filter, thoroughly washed with 95% ethanol, absolute ethanol, acetone and ether. Provided the residue is never filtered to dryness until it has been washed with ether, the product is a fine white powder.

Hydrolysis of the polysaccharide fractions

(1) *Pectin.*—(The following method is a modification of the method described by Jermyn.[19]) The concentrated filtrate and washings are made up to 25 ml. with water. Two 10-ml. portions are pipetted into boiling tubes and a few crystals of urea (about 5 mg.) are added. The tubes are partially immersed in a boiling-water bath and when the contents of the tubes are at bath temperature 0·3-ml. portions of water-white conc. HNO_3 are added dropwise, with shaking, to each tube. The tubes are lightly stoppered. One tube is removed after 3 h. hydrolysis (sugars), the other after 12 h. (uronic acids).

Amberlite I.R.A.-400 (CO_3^{2-}) resin is gently stirred with the 3-h. hydrolysate until the solution is neutral to litmus paper, filtered off, washed well with water and the filtrate plus washings concentrated *in vacuo* at 35° to approximately 2 ml. and diluted to 10 ml. in a volumetric flask with saturated benzoic acid solution.

The 12-h. hydrolysate is transferred to a 100-ml. B.24 flask, Amberlite I.R.A.-400 (acetate) resin added and the stoppered flask mechanically shaken for 2 h. The resin is filtered off, washed with water and extracted in the flask twice with 50 ml. of 2M-acetic acid. The combined filtrates and washings, free from nitric acid but containing the uronic acids, are concentrated *in vacuo* at 35° and made up to 10 ml. with saturated benzoic acid.

(2) *Hemicellulose.*—Hydrolysis of the hemicellulose fraction is carried out by the method of Jermyn.[19] The hydrolysate is de-ionised with Amberlite I.R.A.-400 (CO_3^{2-}) resin as described above.

(3) *α-Cellulose.*—Hydrolysis of the α-cellulose is carried out by the 72% H_2SO_4 method of Monier-Williams[30] as described by Jermyn.[19]

Paper chromatography

The sugars and uronic acids are separated by descending paper chromatography using Whatman No. 1 filter paper using published techniques.[35–37] The positions of the sugars and uronic acids are determined by spraying part of the dried papers with *o*-aminodiphenyl hydrogen oxalate reagent.[18] The sugars and uronic acids are eluted from the unsprayed portions of the papers in 2 ml. of water. The solution is filtered through a 14-mm. diameter, porosity 2, sintered-glass filter and a 1-ml. aliquot containing 10–100 μg. of carbohydrate is taken for the determination of the individual sugars and uronic acid by the ultra-violet spectrophotometric method of Bath.[31]

Application of the analytical scheme and discussion

The proposed scheme was devised to determine the relative nutritive value of the various polysaccharide fractions for ruminants. The results given in Table II for samples of H1 and perennial ryegrass were obtained during the course of digestibility and other studies, to be published in more detail elsewhere, and were not intended as an exhaustive study of the dry matter of the herbage materials. Since the above work was completed, however, Waite & Gorrod[38] have shown that it is possible to identify over 90% of the dry matter of grasses by successive extractions followed by detailed examination of the fractions.

The presence of all of the sugar residues in each of the three fractions emphasises the difficulty experienced in isolating well-defined groups of the structural carbohydrates. However, the fact that 87·4% of the glucose was found in the α-cellulose residue and 86·4% of the

Table II

Composition of the structural polysaccharides of herbages

(% of herbage dry matter)

	H1 Ryegrass/S100 clover	Perennial ryegrass
Pectin		
Glycosyl residue		
arabinose	0·58	0·71
galactose	0·42	0·33
galacturonic acid	0·33	0·43
glucose	1·03	1·18
xylose	0·16	0·15
Hemicellulose		
Glycosyl residue		
arabinose	1·83	2·28
galactose	0·46	1·05
glucose	1·46	1·27
xylose	7·29	7·19
α-Cellulose		
Glycosyl residue		
arabinose	1·02	0·86
galactose	0·18	1·23
glucose	17·24	19·03
xylose	0·99	1·61

xylose in the hemicellulose fraction does indicate that these two components were substantially separated from each other.

By this method of analysis it has been possible to investigate the rate and extent of digestion of the pectin, hemicellulose and α-cellulose fractions of grasses in addition to the study of the rumen fermentation of the individual monosaccharide compounds. The results of the latter investigation have revealed a relationship between the rate of fermentation of the various polysaccharides and their chemical structure.

The proposed method involves a gentle dissolution of the cell-wall preparation, hydrolysis of the dissolved fractions and analysis of the hydrolysates for constituent sugars ; thus the total amount of each polysaccharide is determined. Unfortunately, the analysis is necessarily time-consuming and in its present form is not considered an alternative method for the routine analyses of herbage materials. However, a knowledge of the relative utilisation of different herbage carbohydrates by the ruminant, obtained by this method, is an essential preliminary to the derivation of a simpler technique by which the nutritive value of feedstuffs may be assessed.

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DETERMINATION OF DRY MATTER AND VOLATILES IN SILAGE*

By P. McDONALD and W. A. DEWAR

An apparatus designed to collect the volatile constituents produced during the drying of foods has been used to investigate the losses of volatiles from silages dried at 100°. In 28 silages examined the mean volatility of acetic acid was 87·9% and of butyric acid 89·4%. In all samples, lactic acid was found to be volatile, the percentage volatility ranging from 1·4 to 16·4. Appreciable losses of nitrogen occurred during the drying of silages with high pH values.

Introduction

The determination of dry matter in a feeding stuff is usually carried out by heating a sample overnight in an oven at a temperature about 100°, the loss of weight being considered to be water and the residue to be dry matter. This method cannot be applied to the accurate determination of dry matter in foods containing volatile constituents other than water. Silage comes into this latter category and a number of workers, including Watson & Ferguson[1] and Woodman[2] have drawn attention to the importance of taking into consideration the volatile compounds lost on heating, in order to assess the true losses of dry matter occurring during the ensilage process. Watson[3] has reviewed the nature of these volatile compounds, which include acids, bases and alcohols.

A number of suggestions for determining the 'true' dry matter value of silage have been proposed and one of the most widely accepted methods is to correct the 'apparent' dry matter for volatile fatty acids and nitrogen (calculated as NH_3) lost on drying. The volatile loss is derived from an analysis of the fresh and dried silages.[1, 4, 5] Perkins[6] has described an alternative method based on the Dean & Stark toluene-distillation technique in which the water is

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collected and measured volumetrically. The former method does not take into account the volatility of lactic acid and in the distillation technique the moisture content will be over-estimated unless the aqueous distillate is analysed for volatile constituents.

The purpose of the present investigation was to study the nature of the volatiles lost during the oven-drying of silages by collecting and analysing the distillates produced.

Experimental

Apparatus

The apparatus used for drying the silages and collecting the distillates is shown in Fig. 1 and consists of a round-bottomed, two-necked 1000-ml. flask (A) housed in a thermostatically controlled electric oven. The main outlet from the flask is connected through an insulated stillhead (B) passing through an opening in the oven roof to a Liebig condenser (C) and thence through a long delivery tube (D) into a 300-ml. Kjeldahl flask (F). During a determination the Kjeldahl flask is enclosed in a wide-mouthed vacuum flask containing a mixture of ice and salt at −10°. A gentle current of pre-heated, CO_2-free and moisture-free air is pumped through the side-neck (J) into the two-necked flask containing a weighed amount of chopped silage. The air leaves the apparatus through pre-weighed absorption tubes containing silica gel (G) and soda lime (H). Finally the air is passed through a gas wash bottle containing standard H_2SO_4.

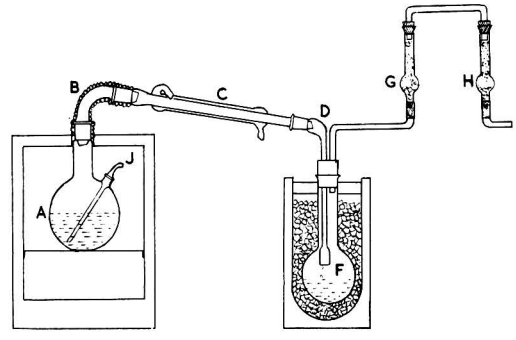

FIG. 1.—*Apparatus used for collection of volatile matter*

Analysis of distillate

Volatile fatty acids (formic, acetic, propionic and butyric) were determined by the column chromatographic method of Wiseman & Irvin ;[7] lactic acid by the ceric sulphate oxidation method of Elsden & Gibson ;[8] total nitrogen and ammonia by the micro-Kjeldahl method and alcohol by Winnick's microdiffusion technique.[9]

Before investigations of silage, recoveries of volatile fatty acids, lactic acid and ammonia were determined in the apparatus described above. During these determinations the oven temperature was maintained at 100° and the rate of air flow kept constant at 0·5 cu. ft./h. In the determination of volatiles in silage a similar drying temperature and air flow were maintained. For this determination about 100 g. of chopped silage were accurately weighed into the distillation flask. On completion of the drying process the silage residue, distillation and absorption tubes were weighed and the distillate was analysed for formic, acetic, propionic, butyric and lactic acids, nitrogen and alcohol.

Results

Recoveries of pure solutions are shown in Table I, and it is clear that within the limits of experimental error, all volatiles were recovered during distillation.

Twenty-eight samples of grass silages, made without additives, ranging in pH value from 3·7 to 5·2 were examined. In all cases the recovery of total volatiles in the receiver and silica gel tube accounted for the losses from the distillation flask. The percentage recoveries (by weight)

Table I

Recoveries of pure solutions in distillates

	Individual acids or ammonium hydroxide, %		Mixture of acids and ammonium hydroxide %	
Acetic acid	99·3	98·8	98·8	99·6
Propionic acid	99·1	99·8	99·2	99·7
Butyric acid	99·3	98·5	98·7	99·4
Lactic acid	67·0[a]	67·7[b]	69·0[c]	70·2[d]
Ammonium hydroxide	95·3	94·8	94·7	93·7

Residual lactic acid after hydrolysis in distillation flask: [a] 32·7%, [b] 32·7% [c] 30·4% [d] 28·3%

ranged from 99·60 to 100·36 with a mean recovery of 99·94% for the 28 silages examined. The CO_2 absorption tube did not increase in weight and ammonia was not detected in the acid wash bottle in any of the determinations. The results of the analysis of fresh silages and distillates are given in Table II. In addition to the ' apparent ' dry matter values, i.e., the residues after drying for 18 h. at 100° in the distillation apparatus, the corrected dry matter values are given and these were obtained by including the total volatiles as determined in the distillates.

Table II

Composition of silages

No.	pH	mg./100 g. of fresh silage								%		
		Nitrogen		Acetic acid		Butyric acid		Lactic acid		Dry matter		$\frac{b-a}{b} \times 100$
		Total	Volatile	Total	Volatile	Total	Volatile	Total	Volatile	Apparent *a*	Corrected *b*	
1	3·7	586	0	343	331	0	0	1827	156	18·60	19·09	2·55
2	3·7	336	7	373	338	32	30	2004	215	16·32	16·91	3·51
3	3·7	478	9	356	342	8	8	2315	191	20·51	21·07	2·66
4	3·8	568	0	345	316	0	0	1660	148	19·29	19·75	2·35
5	3·8	589	0	358	313	0	0	1579	168	18·33	18·81	2·56
6	3·8	346	0	367	348	80	70	1446	93	16·14	16·67	3·18
7	3·8	326	39	259	217	135	123	1717	104	19·58	20·22*	3·17
8	3·9	571	0	345	333	0	0	1661	146	18·80	19·28	2·48
9	3·9	326	6	368	298	213	206	1418	91	14·70	15·30	3·92
10	3·9	379	5	253	218	24	20	1821	128	15·12	15·49	2·40
11	3·9	374	2	234	186	29	23	1744	168	16·42	16·80	2·25
12	4·0	364	2	264	194	29	26	1611	157	61·98	17·34	2·08
13	4·1	297	8	307	284	336	330	825	54	14·07	14·75	4·61
14	4·1	366	7	266	235	30	23	1736	108	15·97	16·34	2·26
15	4·1	345	12	261	238	23	24	2097	98	18·62	19·07	2·36
16	4·3	669	95	298	257	0	0	1989	250	18·22	18·84	3·30
17	4·3	373	44	277	257	67	60	2106	313	35·42	36·13†	1·98
18	4·3	292	7	473	368	27	22	684	112	23·08	23·81**	3·09
19	4·35	647	35	296	275	0	0	1808	216	18·22	18·75	2·84
20	4·5	668	61	397	357	0	0	1914	247	16·93	17·61	3·85
21	4·7	473	31	388	362	380	312	222	3	19·91	20·62	3·44
22	4·7	477	92	315	229	429	424	198	27	23·46	24·35	3·66
23	4·8	229	21	210	168	269	253	1619	11	14·33	14·79	3·11
24	4·9	645	152	319	284	0	0	1615	265	17·57	18·30	3·99
25	4·9	427	12	747	589	96	55	2360	47	17·44	18·14	3·86
26	4·9	253	51	661	551	370	299	20	1	15·54	16·45	5·55
27	5·1	362	133	344	333	661	668	265	11	16·73	17·90	6·56
28	5·2	254	123	555	546	698	629	105	8	13·80	15·13	8·80

* Including 0·15% volatile propionic acid
† Including 0·04% volatile propionic acid
** Including 0·23% volatile propionic acid

Discussion

The volatile N expressed as % of the total N ranged from 0 to 48·4% in the 28 samples examined. A number of workers (e.g., [1, 10, 11]) have commented on the direct relationship between volatile N and pH value and these results tend to confirm this finding. This relationship appears to be better at high pH levels and Smith & Comrie[11] stated that the critical zone occurs about pH 4·5. Unfortunately in the present studies only eight silages had pH values

above this level and only two of these were above pH 5·0. Schoch[4] has shown that losses of volatile nitrogen can be considerable above pH 5·0. At high pH levels there appears to be an inverse relationship between volatile N and lactic acid content, a relationship which would be expected from a knowledge of the fermentation reactions occurring during ensilage. Silages with pH below 4·0 have negligible volatile N contents, with the exception of sample 7. This silage was unusual in containing propionic acid as well as an appreciable amount of butyric acid.

Acetic acid was present in all the silages examined. The volatility of this acid, during the drying process, ranged from 72·7 to 98·4% with a mean value of 87·9% for the 28 silages examined. Watson[3] has studied the volatility of fatty acids in silages and in an examination of 67 silages found that the total volatile acids (calculated as acetic acid) ranged from 50·1 to 90·2% of the total amount present, with a mean value of about 77%. In these experiments, however, the silages were dried at 98° which is slightly lower than the temperature used in the present studies. The total amounts of acetic acid present in silage can be considerable; in this investigation, 18 silages had total acetic acid values above 0·3% of the fresh material. It is clear from the results that this acid is the main volatile component of most silages.

Butyric acid is usually absent or present in only small amounts in well-preserved silages. In the 20 samples examined which had pH values below 4·5, 13 contained butyric acid although in only three cases did the amounts present exceed 0·1% of the fresh silages and two of these samples were associated with exceptionally wet material. The percentage volatility of this acid in the silages ranged from 57·3 to 104 with a mean value of 89·4%. In the two samples of pH value above 5·0, the quantity of volatile butyric acid present was higher than that of any other volatile constituent. This association of high butyric acid content with high pH is well known and is a reflection of the type of bacterial fermentation which has occurred.

Propionic acid was detected in only three silages, in samples 7, 17 and 18. The percentage volatilities of this acid in these samples were 118, 92 and 86 respectively. In view of the unreliability of one of these values and because of the few samples involved, it is impossible to comment on these results.

Small quantities of formic acid have been detected in silages (Langston *et al.*[12]) but this acid was not found in any of the silages examined here.

Lactic acid is usually regarded as being non-volatile but it was recovered in the distillate from all silages. The volatility of the acid was also demonstrated in the preliminary experiments with pure solutions. Smith[13] and Woodman[2] have already drawn attention to the importance of considering this property in silage studies. The percentage volatility of lactic acid in the 28 silages ranged from 1·4 to 16·4 with a mean value of 8·7%. These values are lower than those found for acetic and butyric acids, but because lactic acid is usually present in larger quantities than the fatty acids in well-preserved silages, the losses during drying can be significant. The reason why lactic acid is frequently ignored in dry matter corrections may be due to the difficulties in assessing the volatility of the acid since difference calculations based on analysis of fresh and dried silages are not valid owing to the formation of lactic anhydride and lactide during heating.

Alcohols have been isolated from silage by many workers but not exceeding about 0·5% of the fresh silage (Barnett[14]). Alcohols, as determined by Winnick's micro-diffusion method, were not detected in any of the silages examined.

From a comparison of the apparent dry matter values with the corrected dry matter figures given in Table II, it is clear that the losses of volatiles during drying are of importance in assessing the true dry matter of silages. These differences become more significant in balance experiments where silage losses are calculated. The total volatiles ranged from 1·98 to 8·80% of corrected dry matter and were highest in the silages with high pH containing relatively large amounts of butyric acid and volatile N.

Conclusions

It is clear from an analysis of the distillates produced during the drying of silages that considerable losses of volatiles occur and these must be considered in order to arrive at a true value for dry matter. The high losses of acetic and butyric acids which occur during the drying of silages have been stressed by a number of workers, although the volatile nature of lactic

acid under normal drying conditions is frequently overlooked. The apparatus used in this experiment for the collection of silage distillates can be used for the determination of dry matter although the necessity for analysing the distillate makes the method tedious for routine purposes.

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EFFECTS OF FERTILISERS AND FARMYARD MANURE ON THE COPPER, MANGANESE, MOLYBDENUM AND ZINC REMOVED BY ARABLE CROPS AT ROTHAMSTED

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The Cu, Mn, Mo and Zn contents of five different crops grown in an experiment testing farmyard manure (F.Y.M.) and N, P and K fertilisers were measured. Fertilisers and F.Y.M. had similar and rather small effects on the *percentages* of micronutrients in the crops; the *total* amounts of micronutrients removed were related mainly to the yields. Clover and kale removed much more molybdenum than did the other crops; clover had a high requirement for all the micronutrients examined. The fertilisers used supplied only insignificant amounts of micronutrients, whereas the 15 tons/acre of F.Y.M. supplied as much Cu, Mn and Mo as the five crops together removed and nearly as much Zn. The soil used contains enough of these micronutrients for very many rotations of arable crops provided the total quantities present become available. Although the F.Y.M. dressing used supplied more of each of the micronutrients than was needed by any of the crops grown, its effect on crop growth was due to the N, P and K supplied and to improved physical conditions in the soil.

Introduction

Blood[1] described a permanent field experiment having a large number of small plots to test fertilisers and farmyard manure for several crops; such 'reference plots' are often used to aid advisory work on soil problems as they show the effects of fertilisers on crop composition.

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A smaller experiment of the same pattern was laid down at Rothamsted in 1956 on a rotation of five arable crops to test farmyard manure (F.Y.M.) and also all combinations of fertilisers supplying nitrogen (N), phosphorus (P) and potassium (K). P and K fertilisers increased the yields of all crops and N increased all except clover yields. Fifteen tons per acre of F.Y.M. were more effective than the fertiliser dressings supplied for all crops except kale. As the better yields with F.Y.M. might have been due to the micronutrients it contained, the crops were analysed to see whether those grown with F.Y.M. had more micronutrients than those grown with inorganic fertilisers. There is no collection of information on the amounts of micronutrients in British crops similar to that published in U.S.A. by Beeson.[2] There is also no published British information to show whether dressings of N, P and K affect the micronutrient contents of field crops. The work described here had the secondary purposes of supplying this information and of assessing the rates at which micronutrients are removed by arable crops grown at Rothamsted.

The amounts of copper, manganese, molybdenum and zinc in the crops harvested from the Rothamsted Reference Plots in 1956 are presented here. The effects of manures on crop yields and on N, P and K contents, for a 5-year rotation of the experiment, will be described in a later paper.

Experimental

The experiment was laid out at Rothamsted (Great Field IV) in Spring 1956 on clay-loam soil derived from Clay-with-Flints. The site had grown poor-quality permanent grass for at least 60 years and had received little or no manure or fertiliser in this time. Initially the soil was acid (pH 5·7), and 30 cwt./acre of hydrated lime were given before starting the experiment. The surface layer of turf (2 in. deep) was removed before ploughing for the first crop. The soil was 'very low' in dilute-acid-soluble P and K and contained 0·26% of total N. Wheat, barley, clover, potatoes and kale were grown and each crop occupied one row of plots, individual plots being 7 ft. × 8 ft. Testing all combinations of N, P and K fertilisers required 8 plots and additional plots were included to test F.Y.M. (at 15 tons/acre) alone, and also with the complete dressings of NPK fertilisers. The rates of dressing used were (in cwt./acre):

	Wheat	Barley	Clover	Potatoes	Kale
N (as 'Nitro-Chalk')	0·6	0·45	0·15	0·6	1·0
P_2O_5 (as superphosphate)	0·5	0·5	0·5	0·5	0·5
K_2O (as potassium sulphate)	1·0	1·0	1·0	1·0	1·0

The crops were harvested by hand and oven-dried samples were analysed chemically (after wet oxidation of organic matter with nitric and perchloric acids) by the following methods: Cu by Piper's method with sodium diethyldithiocarbamate,[3] Mn by the periodate method of Willard & Greathouse,[4] Mo by the thiocyanate method of Purvis & Peterson,[5] Zn by the method of Cowling & Miller[6] with dithizone.

Table I summarises yields given by F.Y.M. and the complete fertiliser mixture and Table II the full data for the effects of manures on micronutrient compositions of the five crops.

Table I

Yields of crops grown in 1956 with fertiliser and with farmyard manure in the Rothamsted Reference Plots

	Without fertiliser	With NPK fertiliser	With F.Y.M	With F.Y.M. + fertiliser
	Yields of crops (cwt./acre)			
Wheat				
grain	8	22	30	26
straw	12	30	40	46
Barley				
grain	12	20	34	31
straw	12	18	30	39
Clover (dry hay)	24	48	61	59
Potatoes (tubers)	53	159	190	269
Kale (total crop)	226	431	359	491

Table II

Micronutrient contents of the crops grown on the Rothamsted Reference Plots in 1956
(in parts per million of elements in crops dried at 105°)

Crop	Treatments									
	O	N	P	NP	K	NK	PK	NPK	FYM	NPK + FYM
					Copper					
Wheat										
grain	10	7	11	9	12	8	9	7	11	11
straw	6	10	14	16	5	5	15	6	6	5
Barley										
grain	8	10	8	11	9	13	9	9	12	13
straw	10	7	8	5	6	12	6	10	7	5
Clover										
1st cut	21	21	22	22	24	18	23	21	18	19
2nd cut	19	18	17	18	11	16	22	15	15	20
Potatoes (tubers)	7	6	10	6	10	11	5	10	12	6
Kale (total crop)	5	6	4	5	3	4	3	5	3	7
					Manganese					
Wheat										
grain	61	48	52	61	61	48	61	57	69	70
straw	61	61	69	72	64	52	69	78	87	82
Barley										
grain	22	17	26	17	22	44	26	39	26	35
straw	69	52	70	61	46	96	52	78	88	90
Clover										
1st cut	82	78	87	78	78	82	69	96	87	82
2nd cut	130	163	130	98	78	65	98	78	98	98
Potatoes (tubers)	9	9	9	9	9	13	9	9	11	13
Kale (total crop)	39	44	31	26	26	31	17	44	26	44
					Molybdenum					
Wheat										
grain	0·06	0·06	0·06	0·03	0·06	0·06	0·06	0·03	0·09	0·06
straw	0·13	0·14	0·18	0·10	0·18	0·14	0·14	0·15	0·16	0·09
Barley										
grain	0·21	0·24	0·12	0·19	0·11	0·21	0·12	0·18	0·18	0·21
straw	0·18	0·24	0·15	0·19	0·18	0·22	0·16	0·15	0·09	0·19
Clover										
1st cut	0·74	0·66	0·89	1·03	0·72	0·57	0·64	0·77	0·43	0·30
2nd cut	1·57	2·00	2·54	2·13	1·85	1·65	1·42	1·74	1·09	1·00
Potatoes (tubers)	0·18	0·12	0·18	0·15	0·18	0·15	0·13	0·16	0·12	0·12
Kale (total crop)	0·58	0·81	0·41	0·31	0·56	0·76	0·41	0·44	0·47	0·32
					Zinc					
Wheat										
grain	45	45	42	46	48	45	48	53	53	67
straw	49	44	34	51	29	32	29	42	21	54
Barley										
grain	37	40	30	33	34	42	34	37	32	49
straw	56	30	48	40	38	78	33	44	33	37
Clover										
1st cut	80	72	66	82	56	74	66	60	72	56
2nd cut	72	72	66	76	64	66	61	61	69	53
Potatoes (tubers)	26	26	26	28	28	21	22	21	24	26
Kale (total crop)	37	46	34	41	28	33	33	38	32	30

Discussion

Effects of fertilisers on micronutrient contents of crops

The effects of N, P and K fertilisers and of F.Y.M. on percentages of micronutrients in the crops are summarised in Table III. (The 'effect' of N was calculated as the percentage of each element in the crop with NPK fertiliser minus the percentage in the crop grown with PK only; the 'effects' of P and K were calculated similarly; the effect of F.Y.M. was calculated for manure used alone and also when used with NPK fertiliser.) There were few systematic effects of manurial treatments on the percentages of micronutrients in the crops. N, P, K and F.Y.M. all depressed the percentages of Cu, Mo and Zn more often than they increased them,

presumably because the treatments increased yields and 'diluted' the micronutrients in the harvested crops. Mn contents however were generally raised by all the manurial combinations tested. The only consistent effects on any one crop were that all treatments increased the percentages of Mn in wheat straw and depressed the Zn percentages in both cuts of clover.

Table III

Numbers of positive and negative effects of fertilisers and farmyard manure on the micronutrient contents of crops

	Effect of						Total no. of effects
	N (NPK−PK)	P (NPK−NK)	K (NPK−NP)	NPK alone	F.Y.M. alone	F.Y.M. in presence of NPK (NPK+F.Y.M.−NPK)	
Copper							
positive	3	3	2	2	3	4	17
negative	4	5	5	2	4	4	24
Manganese							
positive	5	5	5	5	6	5	31
negative	2	3	2	2	2	2	13
Molybdenum							
positive	6	4	3	3	2	3	21
negative	2	4	4	5	6	5	26
Zinc							
positive	5	3	3	2	1	4	18
negative	2	4	5	5	7	4	27

Effects of manuring on micronutrients removed by crops

The total amounts of Cu, Mn, Mo and Zn removed by each crop with NPK fertiliser and with F.Y.M. are shown in Table IV. In most of the comparisons all crops (except kale) grown

Table IV

Amounts of copper, manganese, molybdenum and zinc (g./acre) removed by crops grown with NPK fertilisers and with farmyard manure

	Copper		Manganese		Molybdenum		Zinc	
	With NPK	With F.Y.M.	With NPK	With F.Y.M.	With NPK	With F.Y.M.	With NPK	With F.Y.M.
Wheat								
grain	8	17	65	104	0·04	0·14	60	80
straw	8	11	118	178	0·22	0·33	64	44
Barley								
grain	9	21	39	45	0·17	0·31	37	55
straw	9	10	73	132	0·14	0·14	42	50
Clover (2 cuts)	46	52	219	280	2·83	2·05	148	219
Potatoes (tubers only)	18	27	17	24	0·30	0·25	40	52
Kale (total crop)	20	10	173	83	1·75	1·49	150	101

with F.Y.M. removed more micronutrients per acre than were removed by crops having NPK fertilisers, because higher yields were obtained with F.Y.M. (Table I). Kale grown with NPK fertilisers yielded better and removed more of each of the elements examined than kale treated with F.Y.M. Clover generally removed more Cu, Mn and Zn than was taken up by the other crops grown. Both clover and kale removed several times as much Mo as the other crops. The total amounts of micronutrients removed by all five crops grown are shown in Table V for several of the manuring combinations tested. Crops grown with F.Y.M. removed more than did crops grown with NPK fertilisers, again because yields with F.Y.M. were higher.

Spectrographic determinations of some trace constituents in this soil (d.c. arc method) gave the results (p.p.m.): Ba 240, Cu 26, Sr 34, Co 18, Ni 33, V 49, Cr 52, Pb 51. Total contents of Mo, Mn and Zn (determined by chemical methods) were (p.p.m.): Mn 1400, Mo 1·2 and Zn 110. (After fusion of the soil with sodium carbonate, Mo was determined on a hydrochloric acid extract as thiocyanate,[5] Zn by the mixed colour dithizone method[7] and Mn by the method of Willard & Greathouse.[4] Spectrographic analysis of the silica residues remaining after extracting sodium carbonate melts with HCl showed that all of the micronutrients had been extracted.)

Table V

Total amounts of micronutrients removed by five crops grown on the Rothamsted Reference Plots in 1956

	Without manure	With NPK fertiliser	With F.Y.M.	With F.Y.M. + NPK fertiliser
	Amounts of elements in g./acre			
Copper	58	118	148	147
Manganese	334	704	846	1015
Molybdenum	3·0	5·4	4·7	4·5
Zinc	295	541	601	743

Spectrographic and chemical analyses repeated for each block of the experiment, showed that the soil of the site was nearly uniform in micronutrient content. (The levels of Cu, Mn and Zn in the turf top-soil, which was removed before the experiment started, were similar to those in the cropped soil on the experimental site, Mo at 0·5 p.p.m. was less.) Swaine[8] gives the following common ranges (in p.p.m.) for total amounts of micronutrients in British soils: Cu 2–100, Mn 200–3000, Mo 0·2–5, Zn 10–300. On this basis the Mn content of the Reference Plots soil is about average, but the Cu, Mo and Zn data are rather below average.

Assuming the conventional weight of an acre of surface soil (2,000,000 lb.), Table VI compares approximate total quantities of Cu, Mn, Mo and Zn present with the amounts removed by the five arable crops grown in 1956.

Table VI

Approximate total amounts (lb./acre) of micronutrients in soil on Great Field IV at Rothamsted and the amounts removed by five arable crops

	Total amounts present	Approximate amounts removed by five crops grown with F.Y.M. + NPK fertilisers
Cu	52	0·3
Mn	2800	2·2
Mo	2·4	0·01
Zn	220	1·6

If the total amounts present become available to crops, the reserves of micronutrients in the top-soil would suffice for very many years of cropping; the reserves are least with zinc and greatest with manganese. (There is at present no certain method of determining the proportions of the total amounts of micronutrients present in soil that are available to crops.)

Stojkovska & Cooke[9] analysed some of the trace constituents in the F.Y.M. and fertilisers used in this experiment. The supply by common dressings would be approximately:

	Amounts of micronutrients supplied, lb./acre			
	Cu	Mn	Mo	Zn
F.Y.M. (15 tons/acre)	0·5	3	0·01	1
'Nitro-Chalk' (500 lb./acre)	0·01	0·01	0·0005	0·01
Superphosphate (400 lb./acre)	0·02	0·004	0·0007	0·06
Potassium sulphate (200 lb./acre)	0·002	0·002	0·00002	0·002

Comparison with Table VI shows that the amounts of micronutrients supplied by common dressings of the ordinary fertilisers are quite unimportant for replacing soil reserves of these elements; a dressing of F.Y.M. of the quality used, once in the rotation, would replace as much Cu, Mn and Mo as was removed by the crops grown, and nearly as much Zn.

In the first year of this experiment F.Y.M. gave better yields of four crops than did NPK fertilisers; data discussed above show that this did not occur because the crops took up much more micronutrients. The outstanding effects of F.Y.M. were undoubtedly associated with the benefit of organic manuring for crops grown on soil that was shallowly cultivated after ploughing for the first time in very many years. In subsequent years the plots have been hand dug, cultivations have been better, and yields from NPK fertilisers and from F.Y.M. have been similar. Boyd[10] estimates that the effective average contribution of 15 tons of F.Y.M. to crop

nutrition is N 0·45 cwt., P_2O_5 0·6 cwt. and K_2O 1·1 cwt. (The manure used contained N 0·60%, P_2O_5 0·27% and K_2O 1·11%; these are close to data assumed for 'average' quality manure.) The main differences between the NPK fertiliser dressings used and the effective contribution of F.Y.M. (assuming Boyd's data are roughly correct) was therefore that manure supplied less nitrogen to wheat, potatoes and kale than was provided by the fertiliser used. The nutrients supplied by F.Y.M., however, may have been used more efficiently because a better root system was established in the decaying organic manure.

Conclusions

(i) Farmyard manure and a complete NPK fertiliser mixture had similar effects on the proportions of Cu, Mn, Mo and Zn in wheat, barley, clover, potatoes and kale. Fertilisers and also F.Y.M. generally depressed the contents of Cu, Mo and Zn more often than they increased them; Mn contents were generally increased by both fertilisers and farmyard manure. N, P and K had no specific and consistent effects on micronutrient contents of crops that were not associated with the effects of the fertilisers on yields. F.Y.M. supplied more of each of the micronutrients than were contained in the crops grown but the effects of F.Y.M. on micronutrient uptake were similar to those of an NPK fertiliser mixture that supplied almost no micronutrients.

(ii) Individual crops removed varying quantities of micronutrients; clover was outstanding, it removed more of each element than was removed by wheat, barley or potatoes. Both clover and kale removed much more molybdenum than the other crops.

(iii) If the total quantities of micronutrients in the surface layer of this soil become 'available' to crops, these reserves will last for very many rotations. If only a small proportion of any of the elements is 'available', micronutrient deficiencies could occur after several rotations of arable crops grown with ordinary fertilisers and without F.Y.M. Growing crops such as clover and kale, which remove larger amounts of micronutrients, increases the risk of deficiencies. A rotational dressing of 15 tons/acre of F.Y.M. will replace as much micronutrients as are removed, but ordinary fertilisers will not supply worthwhile quantities.

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THE QUALITY OF THE PROTEIN IN GERM AND MILK BREADS AS SHOWN BY THE GROWTH OF WEANLING RATS: THE SIGNIFICANCE OF THE LYSINE CONTENT

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By calculation from data obtained previously, on the growth rate of weanling rats on diets containing white bread as the source of protein supplemented with different amounts of L-lysine, it is now shown that the mean rate of growth is directly proportional to the mean intake of lysine. This applies when the protein content of the diet is 12–13% and the amounts of lysine added are within the range 0–0·25% of the diet. Under these conditions the rate of growth y (g./rat/day) is related to the intake of lysine x (mg./rat/day) by the empirical equation $y = 0{\cdot}06154x + 0{\cdot}03$. Growth rates on bread containing (*a*) wheat germ, (*b*) skim milk powder, and on bread crust have been measured, and the applicability of the equation to these data has been examined. On bread crust the rate of growth was significantly less than on bread crumb. When the observed growth rates were substituted in the empirical equation the calculated lysine content of the crust indicated a loss of available lysine compared with the crumb of about 20%. The observed rates of growth were comparable with the growth rates calculated from the lysine contents of bread containing added raw wheat germ. Heat processing of the raw wheat germ gave slightly lower rates of growth and food consumption, suggesting a small reduction in available lysine due to the processing. Similar results were obtained with bread prepared with dried milk powders (6% of flour weight).

Introduction

Previous work by Hutchinson, Moran & Pace,[1] showed that the growth rate of weanling rats, on diets containing white bread as the source of protein (12–13%), increased when small additions of L-lysine were added to the diet up to 0·2–0·3% of the diet corresponding to a total lysine content of about 0·5%. Up to this level of addition the growth rate was approximately proportional to the quantity of lysine added, and this proportionality has been observed consistently in subsequent experiments. Beyond this level additions of both lysine and threonine were required to obtain further increases in growth rate.[2]

If both the lysine content of the diet and the food consumption are measured the growth rate can then be related to the intake of lysine. From the data obtained on different occasions during the last few years, on the effect of adding lysine to the basic white bread diet, we have now derived the following relationship between the mean lysine intake (x) and the mean rate of growth (y) of male weanling rats:

$$y = 0{\cdot}06154x + 0{\cdot}03 \quad . \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where y = g./rat/day and x = mg./day.

Recently Gupta *et al.*[3] have also found a direct proportionality between weight gain and added lysine when using a basic diet in which the protein was wheat gluten supplemented with methionine, threonine, histidine and tryptophan. Due to the supplementation with these three amino-acids the proportionality between weight gain and added lysine extended over a wider range—from a lysine content (as % of the diet) of 0·27% up to 0·77%. Using their experimental observations the calculated relationship between mean lysine intake (y) and mean rate of growth (x) is

$$y = 0{\cdot}0674x - 0{\cdot}20 \quad . \quad . \quad . \quad . \quad . \quad . \quad (2)$$

which is closely similar to equation (1).

In the present work the effect of supplements of wheat germ and milk powder on the nutritive value of the protein of white bread has been determined by measurement of the rates of growth of weanling rats. The results have been examined, using equation (1), to see how far they can be related to the increased lysine content of the breads brought about by the protein supplements. At the same time some observations have been made on the effects of heat processing on the nutritive value of the two protein supplements.

Experimental

Materials

Flour.—All the flour was milled in the laboratory from Manitoba wheat. The extraction rate of the flour from the wheat was about 67%.

Wheat germ.—Wheat germ was obtained from a commercial mill. Commercial wheat germ is not a standard product but varies in its content of associated endosperm and bran. The N content of the sample used in the experiments given in Table II was 5·32% (dry-weight basis), the lysine content was 6·9 g./16 g. of N. The N content of the sample used in the experiments given in Table IV was 3·6% (dry-weight basis) and the lysine content 6·6 g./16 g. of N.

Milk powders.—In the experiments given in Table V commercial spray-dried skim milk powder (Fison's Milk Products Ltd.) was used. The lysine content (acid hydrolysate) was 8·6 g./16 g. of N. In the experiments given in Table VI the powders were supplied by Dr. W. F. J. Cuthbertson and the lysine contents (on acid hydrolysates by microbiological assay with *Lb. mesenteroides*) were (g./16 g. N) (*a*) normal spray-dried 8·7, (*b*) normal roller-dried 8·0, (*c*) scorched roller-dried 7·2.

White bread.—Bread was made and dried as described previously.[2]

Milk bread.—A mixture of 100 parts of flour with 6 parts of milk powder was used to make bread which was prepared and dried in the same way as the white bread.

Germ breads.—Four germ breads were made containing (*a*) 5, (*b*) 10, (*c*) 15 and (*d*) 20 parts of germ, with 95, 90, 85 and 80 parts of white flour respectively. These breads were made and dried in the same way as the white bread.

Diets

The composition of the diets, which contained 88 parts by weight of bread, was the same as that of the basic diet described previously by Hutchinson *et al.*[2]

Animals and their management

Male weanling rats, each group of the same average initial weights, were used. Their management and the design of the experiments were as described previously, Hutchinson *et al.*[2]

Analytical methods

Nitrogen determinations were made by the Kjeldahl or micro-Kjeldahl method. Lysine was determined on acid hydrolysates of bread, germ and commercial spray-dried milk powder by ion-exchange chromatography by the methods of Moore & Stein[4] with the operating conditions given by McDermott & Pace.[5]

Results

Rate of growth of weanling rats on the crumb and on the crust of white bread

As a convenient first examination of the sensitivity of the growth-response relationship, the rate of growth of weanling rats on bread crumb as a source of protein was compared with that on crust, as it has been shown[5] that there is some destruction of lysine in the crust of bread.

Crumb and crust, separated from standard-sized loaves of white bread, were compared as sources of protein in the basic diet. Each diet was supplemented with 0·08% of L-lysine to give better rates of growth than would be obtained at the zero point of the response curve. The results are given in Table I.

These results show a significant reduction in the nutritive value of the protein in the crust of bread as compared with that of the crumb, most probably due to reduction in the available lysine in the crust. From equation (1) the observed growth rate of crumb is equivalent to a daily intake of about 29 mg. of lysine while that on crust is equivalent to one of about 20 mg. of lysine. Calculation from these values (taking into account the respective food intakes and that 0·08% of L-lysine was added to each diet) gives the lysine content of the crumb as about 0·30% and that of the crust about 0·23%—indicating a loss of about 20% in the crust. These

Table I

Growth of weanling rats on diets with bread crumb and bread crust as the source of protein; both diets supplemented with 0·08% L-*lysine*

(12 rats/treatment over 22 days)

Dietary protein	N content of diet, %	Mean weight gain, g./rat/day	Mean food intake, g./rat/day	Weight gain (g.) per g. of N consumed
Bread crumb	2·22	1·87	7·95	10·6
Bread crust	2·20	1·23	6·75	8·2
Standard Error of the mean	(18 d.f.)	±0·04	±0·14	

figures may be compared with the values of 0·29% and 0·24% found analytically for hydrolysates of crumb and crust by McDermott & Pace.[5]

Effect of addition of wheat germ to white flour

These experiments were concerned with the effect on the growth of young rats, of adding wheat germ of ordinary commercial quality to white flour at a number of levels within the range which would be expected in commercial germ or brown bread. The results, which are given in Table II, in conjunction with the data on the lysine response have enabled us to examine the relation of the beneficial effect of a wheat germ supplement to its content of lysine.

The response in the rate of growth to these additions of wheat germ is similar to that observed when lysine is added to a basic white bread diet. The curve for growth response

Table II

Rate of growth of weanling rats on bread made from flour containing commercial wheat germ

Wheat germ in bread, %	N content of diet, %	Mean weight gain, (g./rat/day)	Mean food intake (g./rat/day)	Weight gain (g.) per g. of N consumed
0	2·19	0·95	6·7	6·5
5	2·34	1·81	9·5	8·1
10	2·46	2·48	10·5	9·6
15	2·64	3·26	11·4	10·9
20	2·74	3·29	10·7	11·2

This table is compiled from two separate experiments. In the first 0% and 20% germ breads were compared with 7 rats/group giving for S.E. of the means (13 d.f.) ± 0·09 g. for wt. gain and ±0·21 g. for food intake. In the second experiment the 5%, 10% and 15% germ breads were compared with 8 rats per treatment giving S.E. of the means (16 d.f.) ± 0·08 g. for wt. gains and ±0·22 g. for food intake.

to lysine reaches a plateau when the added lysine is about 0·25%, that is when the total lysine content of the diet is about 0·50–0·55%. With the curve for the response to germ there is a similar flattening out between the 15% and 20% levels of addition of germ and it is noteworthy that this is where the total lysine (from flour and germ) is about 0·5%. It has been shown by Hutchinson *et al.*[2] that when the lysine content reaches about 0·5%, with about 12% bread-protein in the diet, appropriate additions of both lysine and threonine are needed to obtain a further increase in growth rate.

The approximate lysine intakes, on the first four diets of Table II where the growth response is still linear, have been calculated from the analytical figures for white bread and the sample of germ. These values have been substituted in equation (1) and the estimated rate of growth calculated. It will be seen from Table III that these correspond reasonably well with the growth rates observed. Table III also includes the growth rates calculated from equation (2), derived from the data of Gupta *et al.*[3]

Thus the improvement in growth rate brought about by the protein of wheat germ *at these levels of addition* can be accounted for by the lysine contributed by the germ.

Table III

Comparison of growth rates observed on diets of bread containing different amounts of wheat germ with those estimated by calculation from the intake of lysine

Wheat germ in bread, %	Approximate mean lysine intake, mg./rat/day	Observed growth rate from Table II (g./rat/day)	Calculated growth rates (g./rat/day) from regression equation	
			1	2
0	16	0·95	1·0	0·9
5	31	1·8	1·9	1·9
10	43	2·5	2·65	2·7
15	55	3·25	3·4	3·5

Effect of commercial heat treatment on wheat germ when used as a protein supplement to white flour

The experiments given in Table II were carried out with unheated (raw) wheat germ. In the preparation of some proprietary germ flours the germy stock, which is subsequently added to flour, is sometimes heat-treated to stabilise it and to produce a flavour which is considered more attractive than that of raw germ. No precise details of the heat treatments used nor to what extent they vary from plant to plant were available but samples of germ were obtained from one plant before and after the heat treatment. For the feeding trials 10 parts of germ were mixed with 90 parts of white flour and bread made from the resulting meal. Rates of growth on the bread containing the raw germ were compared with those on the bread containing the germ which had received the commercial heat treatment. The results are given in Table IV.

Table IV

Effect of heat treatment on wheat germ used as protein supplement to white flour

(rate of growth of male weanling rats measured over 28 days; 8 rats/treatment)

Dietary protein	N content of diet, %	Mean weight gain, g./rat/day	Mean food intake, g./rat/day	Weight gain (g.) per g. of N consumed
Bread from meal containing 10% raw germ	2·47	2·20	9·2	9·6
Bread from meal containing 10% heated germ	2·46	1·85	8·5	8·8
Standard Error of the mean (10 d.f.)		±0·06	±0·17	

This particular heat treatment has caused a very small reduction in the biologically available lysine in the wheat germ. By substituting the observed growth rates in equation (1) the calculated lysine intake on the ' raw ' germ bread was about 35 mg./rat/day and on the heated germ bread about 29·5 mg./rat/day. In conjunction with the figures for food consumption these values give an estimated lysine content of 0·40% for the ' raw ' germ bread and 0·36% for the bread containing heated germ, a reduction of about 10%.

Effect of addition of dried skim milk to flour

In Britain the consumption of milk bread is only a very small proportion of the total bread consumed, but there is an increasing interest in bread of higher protein content and it is desirable that data should be available on the order of magnitude of the improvement in protein quality by the addition of dried skim milk. For reasons largely connected with baking technology the maximum amount of dried skim milk added in this country is of the order of 6 parts to 100 parts of flour. The effect of this level of addition of dried skim milk upon the rate of growth of weanling rats has been examined and compared with the lysine response relationship.

The first experiments were carried out with an average commercial sample of spray-dried skim milk and this was examined on three separate occasions. The results of these three experiments are given in Table V.

Table V

Growth of weanling rats on white bread and milk bread

(Milk bread made from 100 parts flour + 6 parts dried skim milk)

	Dietary protein	N content of diet, %	Mean weight gain, g./rat/day	Mean food intake, g./rat/day	Weight gain (g.) per g. of N consumed
Experiment 1					
7 rats/treatment	Control bread	2·19	0·95	6·65	6·5
21 days	Milk bread	2·43	2·09	9·2	9·3
Standard Error of the mean	(13 d.f.)		±0·09	±0·21	
Experiment 2					
12 rats/treatment	Control bread	2·22	1·08	7·4	6·5
21 days	Milk bread	2·41	1·74	8·65	8·3
Standard Error of the mean	(18 d.f.)		±0·11	±0·16	
Experiment 3					
8 rats/treatment 28 days	Milk bread	2·41	1·82	8·8	8·6

From these three experiments the mean value for the rate of growth on milk bread was 1·88 g./rat/day. From equation (1) this corresponds to a mean daily intake of 30 mg. of lysine. Calculation from this value, in conjunction with the mean food consumption, gives an estimated lysine content in the milk bread of about 0·35%. The value calculated from the analytical data on hydrolysates of bread and the milk powder is approximately 0·36%. It is clear therefore that the effect of the milk powder protein in improving the growth-promoting quality of white bread may be accounted for by the lysine it contributes.

Comparison of skim milk dried in different ways

Three samples of skim milk powder, (1) normal spray-dried, (2) normal roller-dried, (3) purposely overheated, viz., scorched roller dried, supplied by Dr. W. F. J. Cuthbertson, were examined. These were selected from a series of samples which he is analysing for total lysine and for 'available' lysine determined chemically by the dinitrofluorobenzene method of Bruno & Carpenter.[6]

Milk bread was made from 100 parts of flour + 6 parts of each of these samples of dried milk powder. The breads were then compared as sources of protein for the weanling rats, together with bread made from the commercial spray-dried powder used in the experiments of Table V. The results are given in Table VI.

Table VI

Comparison of milk breads made from skim milk powder dried in different ways

(8 rats/treatment; growth measured over 28 days)

Milk powder in bread	N content of diet, %	Mean weight gain, g./rat/day	Mean food intake, g./rat/day	Weight gain (g.) per g. of N consumed
Normal spray	2·42	1·82	8·7	8·6
Normal roller	2·41	1·70	8·3	8·5
Scorched roller	2·39	1·29	7·5	7·2
Commercial spray*	2·41	1·82	8·8	8·6
Standard error of the mean	(21 d.f.)	±0·06	±0·20	

* The data for this bread are also quoted in Table V

The growth response is similar for breads containing normal roller-dried and spray-dried milk powders and is in good agreement with the data given in Table V for the commercial spray-dried product. The growth response method reveals the heat damage suffered by the scorched roller-dried powder. On the bread containing this powder both rate of growth and food consumption are significantly less than on the breads containing the powder prepared in a normal way. Substitution of the observed rate in equation (1) shows that the calculated intake of lysine on the diet containing scorched roller powder was about 20 mg./rat/day as against about 30 mg./rat/day on the diets containing normally prepared milk powders.

Discussion

The relationship established here between the rate of growth of young rats and their intake of lysine, when the protein of the basic diet is derived from bread, has provided a basis for testing whether the improvement produced in the nutritive value of the protein of bread by small additions of good-quality protein could similarly be ascribed to the lysine contributed by these supplements.

The experiments reported show that improvement in the protein quality of white flour or bread by the addition of up to 15% of wheat germ and of 6% skim milk powder can be accounted for on the basis of their content of lysine. That is, no significant effect upon the growth rate was observed due to the other amino-acids supplied by these protein supplements at the levels they were added. Howard *et al.*[7] found similarly that the beneficial effects of small additions of lactalbumin, soya protein and skim milk powder to white flour could be entirely attributed to the lysine these proteins supplied.

These observations with proteins are similar to and support those previously made on the addition of free amino-acids to a basic white bread diet of protein content 12–13%.[2] Then it was found that with lysine additions up to about 0·25–0·3% there was no further improvement in growth rate when threonine or methionine was also added. Only at a higher lysine addition (0·5%) was there a further response when threonine was added ; the levels of addition of wheat germ and skim milk powder used in the present experiments did not raise the lysine content sufficiently high for a threonine response to be manifest.

Thus 6% of skim milk powder in the bread provided about 0·16% of lysine and 5%, 10% and 15% of wheat germ in the bread about 0·09%, 0·18% and 0·28% of lysine respectively (all as percent of the diet). That is, the additions were in the range where lysine was still the limiting amino-acid.

Again, since growth response was limited by and proportional to the intake of lysine, it may be concluded that the reduced rate of growth on the heat-treated germ and on the severely heated roller-dried milk powder was primarily due to a reduction in available lysine as a consequence of the processing treatments. The growth-response method using the basic diet of white bread therefore provides a method capable of indicating, in some instances of heat processing, whether the treatment has reduced the biologically available lysine.

Finally this work suggests that, when bread is the major source of protein in the diet, the improvement in the nutritive value of its protein brought about by other protein foodstuffs present, will in the first instance be proportional to the available lysine these contain. Some examples of different types of foodstuffs with a relatively high content of lysine are given in Table VII (sources : Harvey[8], McCance & Widdowson[9] and unpublished work in this laboratory).

Table VII

Lysine contents of foods

Foodstuff		Approximate lysine content, g./100 g.
Dried skim milk	(N = 5·69%)	3·0
Cheddar cheese	(N = 4·1%)	2·05
Wheat germ	(N = 4·75%)	2·05
Roast beef	(N = 3·5%)	1·8
Beef tongue	(N = 3·20%)	1·6
Cooked ham	(N = 2·58%)	1·4

It must be emphasised that this work has been restricted to a study of the value of the protein of wheat germ and skim milk powder when used as supplement for bread, and has not been concerned with other nutrients contained in these materials.

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DETERMINATION OF ORGANIC FLUORINE RESIDUES IN BLACKCURRANTS

By H. EGAN and R. WOOD

A method, less time-consuming than that previously proposed, is described for the determination of organically bound fluorine insecticide residues in plant material.

Introduction

Thompson[1] has described methods for the determination in plant material of fluoroacetamide and fluoroacetic acid derived from fluoroacetamide treatment for pest control. Fluoroacetamide can be determined as such or, after alkaline hydrolysis, as fluoroacetic acid: these methods differ only in the manner of extraction and chromatographic treatment and both are time-consuming.

The toxic action of fluoroacetate is due to its enzymic conversion in the tissue to fluorocitrate, which effectively blocks the Krebs tricarboxylic acid cycle.[2] Fluoroacetamide is less toxic to mammals than is fluoroacetate, the respective oral LD_{50} values being 15 mg./kg. and 1–2 mg./kg. for rats;[3] this is thought to be due to the relatively slow hydrolysis *in vivo* of fluoroacetamide to fluoroacetate. Little is known of the fate of fluoroacetamide in plant material, although Thompson recovered at least 70% unchanged fluoroacetamide from field beans after 5-h. absorption through the cut tap roots; he concluded that for residue work it is probably advantageous to hydrolyse fluoroacetamide to fluoroacetic acid and to determine

the two substances together as fluoroacetate. The present work describes a shorter procedure, based on Thompson's method but without loss of precision, for the determination of organic fluorine residues of pesticidal origin in blackcurrants.

Experimental

Basically, the method for the determination of fluoroacetic acid[1, 4] consists of aqueous extraction of the plant material, ether extraction of the fluoroacetic acid from the aqueous extract, removal of any inorganic fluorine present on a silicic acid column, ignition of the organic fluorine compounds with lime and distillation of the released fluoride followed by colorimetric determination of the fluoride ion. In the present study the duration of the ether extraction was reduced from 12 to 8 h. without loss of accuracy, whilst two short ignitions of 20 min. each instead of double ignition with lime originally requiring 7 h. gave satisfactory recoveries with pure fluoroacetic acid. A single fluorine distillation, with collection of less distillate at a higher distillation temperature, replaces the original double distillation without introducing interference in the final colorimetric determination. An attempt to replace the lime ignition by oxygen combustion in a closed flask[5] (which could subsequently be used for fluorine distillation without further transference) was found to be impracticable only on the grounds of difficulty of concentrating the column eluate into a sufficiently small volume. The colorimetric procedure of Megregian,[6] based on bleaching by the fluoride ion of the coloured zirconium Eriochrome (Solochrome) Cyanine R complex, was used since, unlike other available methods,[7] the colour reaction is rapid.

Method

A 50-g. sample of macerated blackcurrants is placed in a 250-ml. stoppered flask and rendered alkaline with 40 ml. of N-NaOH, the mixture shaken for 15 min. and then gently refluxed for 2 h. The procedure described by Ramsey & Clifford,[4] as applied by Thompson, up to and including the chromatographic stage is then followed, with the omission of the phosphotungstic acid precipitation and with reduction of the ether extraction time to 8 h. One g. of fluorine-free calcium oxide is added to the aqueous extract of the column eluate contained in a platinum basin and the solution evaporated to dryness and ashed at 600° for 20 min. When cool, the sides of the basin are washed down with a little distilled water and the solution again taken to dryness and ashed at 600° for 20 min. When finally cool, 15–20 ml. distilled water are added to the white residue followed by 3 ml. of 60% perchloric acid added dropwise with stirring. When effervescence ceases the solution is transferred with washings to a distillation flask, the basin rinsed with a further 20 ml. of 60% perchloric acid and this also added to the flask followed by 1 ml. of 100% w/v silver perchlorate solution and a few glass beads. The distillation apparatus is assembled, the temperature of the flask raised to 120° and steam passed; the distillate, collected in a beaker, is kept constantly alkaline to phenolphthalein by small additions of 0·1N-NaOH. When the temperature reaches 135° a further 150 ml. of distillate are collected, the temperature being maintained between 135° and 140°. The slightly alkaline distillate is then evaporated to less than 100 ml. (to less than 50 ml. if under 25 μg. of fluorine is expected), cooled, a 50-ml. aliquot (or an aliquot diluted to 50 ml.) placed in a measuring cylinder and the fluoride content determined by the method of Megregian.[6] In the present work a Unicam SP 600 spectrophotometer was used; solutions were measured against a piece of coloured glass having a transmission of approximately 1% at 525–530 mμ in order to enable 0–50 μg. of fluorine to be measured on the most sensitive part of the optical density scale. An optical density reduction of 0·012 per μg. of fluorine, equivalent to 0·08 p.p.m. of fluoroacetamide on a 50-g. sample, was obtained.

Results

Fluoroacetamide was added to 50-g. samples of blackcurrants known to be free from pesticide treatment. The residues found by the method described are shown in Table I and compare satisfactorily with those obtained by the longer procedure.

Table I

Recovery of fluoroacetamide added to blackcurrants

Fluoroacetamide added, p.p.m.	Fluorine found, as fluoroacetamide, p.p.m.	% Recovery
0·00	0·00, 0·06, 0·05, 0·02, 0·06	—
0·47	0·50	106
0·58	0·59	102
1·16	0·93	80
1·85	1·65	89
3·5	3·4	97
4·65	3·95	85

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APPLICATION OF DIRECT PHOTOMETRY TO AGRICULTURAL ANALYSIS*

By R. O. SCOTT

The applications of two direct-reading spectrometers (porous-cup spark method) to the analysis of agricultural materials are described: (1) a two-channel instrument for the determination of 0·3–24 p.p.m. of magnesium in acetic acid and ammonium acetate extracts of soils and HCl extracts of plant materials; a coefficient of variation in direct analysis of about ±2·0% is found. The major composition of agricultural samples does not affect the determinations; (2) the eleven-channel Hilger Medium Direct Reader for the determination in soil extracts of 0·1–24 p.p.m. copper, 1–400 p.p.m. manganese and 1–50 p.p.m. zinc, the coefficients of variation being ±1·10%, ±1·64% and ±7·01%, respectively. A tentative pelleted rotating disk method is given for the determination of zinc, boron, manganese and copper in ashed plant materials with coefficients of variation of ±1·9%, ±2·8%, ±3·3% and ±5·4%, respectively.

Introduction

Spectrographic methods are widely used in agricultural laboratories for the determination of elements such as potassium, sodium, calcium and magnesium, and also for that of trace

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elements, for example, copper, iron, manganese and boron. Direct photometry is the modern development of these spectrographic methods, and utilises photoelectric detection and electronic measurement of the spectral line intensities, in place of the photographic plate. One great advantage of a direct photometric method over a spectrographic method is the speed of analysis, but the capital cost of the equipment is much higher, and it can only be justified if a large number of samples have to be analysed.

Flame photometry is the simplest form of direct photometry, and in this method an instantaneous meter reading of a spectral line intensity can be used, since both a homogeneous mist of a solution sample and a stable flame source are employed. The sensitivity is such that only sodium, potassium and calcium can be conveniently determined in agricultural samples. Magnesium has occasionally been determined, but for this element the method is unsatisfactory, since the only magnesium line with sufficient sensitivity at 2851 Å lies on an interfering —OH band system.

Atomic absorption flame spectrophotometry is another form of direct photometry and may be suitable for the determination of elements such as magnesium, copper and zinc in agricultural materials. Variation of phosphorus and aluminium in the samples affects the intensities of the spectral lines in a similar manner to that occurring in flame photometry.

At the Macaulay Institute, sodium, potassium and calcium are determined by flame photometry with a three-channel instrument,[1] while magnesium is determined in the same extract by direct photometry,[2] with a simple two-channel instrument and excitation by a high-voltage spark. A high-temperature spark source, such as that used for the determination of magnesium by direct photometry, produces more complex spectra than a flame, and a prism or grating is essential to resolve the spectral lines. In addition, a spark source is less stable, and the spectral radiations must be integrated over a period of time to average out the fluctuations that occur.

Many commercial direct-photometric instruments are available, varying from the relatively low-priced and low-dispersion, eleven-channel Hilger Medium Direct Reader, to the high-dispersion 30- to 60-channel instruments costing five to ten times as much. An example of the latter type is the Hilger three-metre grating Polychromator. For agricultural materials, a low-dispersion instrument is only of value for the analysis of samples with low iron contents, otherwise a high-dispersion instrument is required to resolve the trace element lines from those of iron.

To illustrate some agricultural applications of direct photometry, the uses of two types of integrating instruments are described. Firstly, a laboratory-made direct-reading attachment to a 20-cm. focal-length Hilger small quartz spectrograph for the determination of magnesium only, and, secondly, the commercial direct-reading attachment for the 60-cm. focal-length Hilger medium quartz spectrograph with which up to 11 elements can be determined.

Determination of magnesium in solution by direct photometry

Apparatus

The direct-reading attachment, which has been described in detail elsewhere,[2] is fitted in place of the plate-holder mounting of an E484 Hilger small quartz spectrograph. Two optical channels are provided, one for the magnesium line at 2802 Å and the other for a strontium internal standard line at 4077 Å.

Two narrow exit slits allow the light from these lines to pass on to photo-multiplier tubes whose output currents charge capacitors. After an exposure, the voltages developed in the capacitors are proportional to the integrated intensities of the magnesium and strontium spectral lines, and are measured by a null-reading electrometer bridge circuit. The maximum voltage which can conveniently be read with the valve-voltmeter is about 8 V, and, should the solution tested give too high a magnesium capacitor voltage, a range extension circuit is used. This consists of an extra capacitor which is switched in in parallel with the normal magnesium capacitor, the voltage being lowered thus to a readable value without the necessity for a second exposure.

Operating conditions

The porous cup electrodes used for the determination of magnesium, and also of copper, manganese and zinc, by a method described later, are made from 5·5-mm. carbon rods, grade SG-905-J (Ship Carbon Co. of Great Britain Ltd.). The cups are approximately 16 mm. long, with bore of 3·2 mm. ($\frac{1}{8}$ in.) diameter, and base thickness of 0·60 $\pm$ 0·01 mm. To improve porosity, they are heated at 450° for 2 h. before use and should remain usable for at least a week. The counter electrodes are prepared from the same grade of carbon as the cups and have sharp points of 70° included angle.

The electrodes, with a spark gap of 2 mm. between the cup and the lower counter electrode, are placed 20 cm. from the entrance slit of the spectrograph, no condensing lens being employed. The source is a relatively weak undamped spark of 15,000 V with 0·02 mH inductance, 0·001 μF capacitance and no added resistance. Strontium is employed as the internal standard, and both sample and standard solutions are prepared containing 500 p.p.m. strontium and 2·0 or 2·25% of acetic acid.

To make a determination, the porous cup is filled with solution from a fine-pointed pipette, care being taken that no air bubbles are trapped. The spark is then started, and the solution seeps through the base of the cup and is excited by the discharge passing between the moist base and the counter electrode. Sparking is stopped after 56 sec. by a time switch, during which period about 0·1 ml. of solution is consumed. The magnesium and strontium capacitor voltages are then measured, and the magnesium content of the solution evaluated from a standard curve. In this way, magnesium can be determined in solution from 0·3 to 6·0 p.p.m., and up to 24 p.p.m. when the range-extension circuit is used.

Effect of variable factors

Negligible errors result from any slight variations in base thickness of the cups or in counter electrode shape, which may occur during their preparation. The position of the electrodes on the optical axis and the length of the spark gap are not very critical, and since an optical projection system with a 15× magnification is used to position the electrodes, no errors result from any small misalignments.

The spectrograph and attachment are in a room with no temperature control. Day-to-day changes in temperature over a 10° range have not caused curve drift, and it would appear that the instrument is fitted with exit slits which are sufficiently wide to take care of any normal temperature change in the surroundings.

Variation in the major composition of soil or plant extracts has been shown[2] not to affect the determination of magnesium. The ratio of the permissible concentration of an extraneous element to that of magnesium may be as high as 40 for aluminium, and at least 300 for potassium, calcium and phosphorus.

Application to agricultural materials

Because of the high sensitivity and the small effect of extraneous elements, magnesium can be determined in solutions derived from practically any type of agricultural material. At the dilution required, limestone, for example, contains no more than 150–200 p.p.m. of calcium in the final solution, an amount that has little, if any, effect on the results. At the Macaulay Institute most determinations of magnesium made are in acetic acid or ammonium acetate extract of soils, and in HCl extracts of ashed plant materials.

Acetic acid extracts of soils.—Ten g. of soil are shaken for 2 h. with 400 ml. of 2·5% acetic acid, and the suspension filtered through a magnesium-free filter paper. Part of the extract is used for the determination of phosphorus colorimetrically, and part, without further concentration, for the determination of sodium, potassium and calcium by flame photometry.

For the determination of magnesium 5 ml. of strontium chloride solution (containing 0·5% of strontium) are diluted to 50 ml. with the acetic acid extract. Standard solutions are prepared by diluting 5 ml. of strontium chloride solution to 50 ml. with stock solutions containing from 0·3 to 24 p.p.m. of magnesium in 2·5% acetic acid. These solutions are treated as described above and the magnesium to strontium intensity ratios calculated.

The standard curve covers from 1·2 to 24 mg. of magnesium per 100 g. of soil, and can be extended to 96 mg. per 100 g. by use of the range-extension circuit described above. Samples with magnesium contents above 96 mg. of magnesium per 100 g. of soil are diluted with a solution containing 500 p.p.m. of strontium in 2·25% acetic acid.

Ammonium acetate extracts of soils.—The exchangeable magnesium content of a soil is determined by leaching 20 g. of soil to 1 l. with neutral normal ammonium acetate solution. Five ml. of strontium chloride solution and 1 ml. of glacial acetic acid are diluted to 50 ml. with the extract. Acetic acid is necessary to ensure percolation through the base of the porous cup. Standard curves are prepared from solutions containing magnesium equivalent to 0·14–11 mg.-equiv./100 g. of soil, and 500 p.p.m. of strontium in a base solution of N-ammonium acetate and 2% acetic acid. Should samples have higher magnesium contents than this, 5 ml. of strontium solution, 1 ml. of glacial acetic and an aliquot of the extract are diluted to 50 ml. with ammonium acetate solution.

Plant materials.—Ten g. of ground plant dry matter are ashed at 450° and, after removal of silica by evaporation with HCl, the filtrate is diluted to 500 ml. with distilled water. Part of this extract is used for the flame photometric determination of sodium, potassium and calcium, and part for the colorimetric determination of phosphorus. For magnesium, 2 ml. of the extract and 5 ml. of strontium solution are diluted to 50 ml. with 2·5% acetic acid to cover a range of 0·04–3·0% of magnesium in the plant material. Standard curves are plotted as before from standard solutions in acetic acid, and incorporating potassium dihydrogen phosphate, potassium sulphate, calcium carbonate and sodium chloride in proportions corresponding to an average plant ash.

Reproducibility and accuracy

The coefficient of variation for a single determination is about ±2%, this value being derived from 40 replicate determinations using a solution containing 1·8 p.p.m. of magnesium.

To assess the accuracy of the method, 20 samples of turnips were analysed in duplicate by both the direct photometric method and by a colorimetric procedure with Titan yellow.[3] The mean values for magnesium were similar, 0·0687% and 0·0685% respectively, but the coefficient of variation found for the direct photometric method, ±2·4%, was better than that for the colorimetric procedure, ±5·6%.

The Hilger direct-reading attachment to the medium quartz spectrograph

Before deciding whether a particular direct reader is suitable for the determinations required, it is necessary to consider the wavelength separations of the available spectral lines. In the Hilger 11-channel direct-reading attachment to the medium quartz spectrograph the choice of the elements to be determined and the spectral lines to be used is restricted by the limitation of the minimum distance between the lines to 4 mm. This attachment can be fitted to any flat-field Hilger medium quartz spectrograph.

In the attachment, exit slits are sited in the focal plane of the spectrograph at the positions of the spectral lines used. The spectral radiations passing through the slits are reflected by mirrors to 11 photomultiplier tubes, the output currents of which charge capacitors, whose voltages, at the end of an exposure, provide meter readings which are measures of the spectral line intensities.

In choosing the most appropriate lines for the Hilger Medium Direct Reader at the Macaulay Institute, consideration was first given to the possibility of including the trace elements of greatest biological importance, such as zinc, boron, iron, magnesium, manganese, copper, cobalt and molybdenum. It was decided that all but the last two could reasonably be expected to give adequate sensitivity and separation with a medium direct reader. Next, two possible internal standard elements, lithium and chromium, were included, and the three additional channels used for phosphorus, aluminium and silicon. An exit slit at a strontium wavelength was also provided as a possible alternative internal standard, but has not so far been allocated to a photomultiplier tube outlet. The wavelengths of the spectral lines employed, along with their separations at the focal plane of the spectrograph, are given in Table I. Two of the elements, lithium and chromium, are normally used as internal standards.

In agricultural materials many of the chosen elements produce spectral lines having low intensities resulting in low output currents from the photomultipliers, so that tubes with low dark currents must be chosen. Determinations at low concentration levels may even then require a correction for dark current, which may vary somewhat from day to day.

A simple adjustment to compensate for small changes in the temperature of the surroundings is incorporated, and controls are provided for varying the sensitivity of each element. Two methods of making a determination can be used. One is by a timed exposure in which pre-spark and exposure times are predetermined by the settings of the appropriate process timers. The other is by an automatic exposure, by which the current output from the photomultiplier of an internal standard charges a capacitor to a constant voltage, whereupon the exposure is automatically stopped.

To make a determination, the appropriate direct-reader and source conditions are selected and the attachment adjusted for temperature. The electrodes are placed in the discharge stand and the start-button pressed, after which all the operations are automatically controlled. The capacitors are first discharged and then the source started. During the pre-spark period the photomultiplier output currents discharge to earth, but are automatically connected to the capacitors when the exposure starts. At the end of the exposure the capacitors are isolated, the source automatically switched off, and the voltages of the capacitors measured in turn on the meter or pen recorder.

Applications of the Hilger Medium Direct Reader

Two methods of excitation are in use at present, a porous-cup solution spark technique for the determination of copper, manganese and zinc in soil extracts, and a pelleted rotating disk method for the analysis of plant materials.

Porous-cup solution spark excitation for soil extracts.—The porous cup electrodes are similar to those previously described for the determination of magnesium, but the counter electrodes have a 70° included-angle point blunted flat to approximately 1 mm. diameter, and are prepared from 5-mm. diameter Ship Carbon rods, SG-305-H. The electrodes are placed 20 cm. from the entrance slit of the spectrometer with a spark gap of 2 mm., no condensing lens being employed.

The source, selected from a B.N.F. type unit,[4] is an uncontrolled spark of approximately 6000 V, with 0·5 mH inductance, 0·005 μF capacitance and no added resistance. These parameters were chosen to produce a relatively weak damped spark, giving minimum background near the copper line at 3247 Å.

Standard and sample solutions are prepared in 4% (v/v) nitric acid, and contain 250 p.p.m. of lithium and 65 p.p.m. of chromium as internal standards. These solutions are sparked using automatic exposures with lithium as the controlling element, sensitivity settings being selected to give an exposure time of 30–35 sec.

At maximum sensitivity and with the photomultiplier tubes at present installed, the lowest levels which can be determined in solution are about zinc 1·0, phosphorus 10·0, iron 0·4, magnesium 0·2, manganese 0·2, aluminium 3·0 and copper 0·1 p.p.m. The limits for boron and silicon have not been determined, as the carbon electrodes employed so far contain small amounts of these elements.

For several of the elements the coefficients of variation with lithium as the internal standard element are given in Table II, the results being derived from ten replicate determinations. Satisfactory coefficients of variation are generally found. The amounts, in p.p.m., of copper, manganese and magnesium extractable from a soil are of the same order as those shown in Table II, while that of zinc is lower.

Table III shows the percentage errors in the apparent contents found in a solution containing copper 1, manganese 10 and zinc 10 p.p.m. in the presence of varying amounts of calcium or potassium with chromium and lithium as the internal standard elements. The amounts of calcium and potassium present in the final solutions prepared from EDTA, ammonium acetate and acetic acid extracts of Scottish soils are normally 100–1500 and 50–200 p.p.m., respectively, and are seldom as high as 4000 p.p.m. and 800 p.p.m., respectively. Variations in the amounts of calcium and potassium at the levels encountered in soil extracts, produce only small errors in the determination of copper and zinc, when lithium is used as the internal standard element,

Table I

Wavelengths of spectral lines employed for general soil and plant work with the eleven-channel Hilger Medium Direct Reader, and the separation of the exit slits

Element	Wavelength, Å	Separation of exit slits, mm.
Zn	2138·6	
		>20
Si[a]	2435·2	
		7·50
B	2497·7	
		6·20
P	2553·3	
		12·49
Cr	2677·2	
		7·01
Fe	2755·7	
		3·91
Mg	2802·7	
		11·00
Mn[b]	2949·2	
		8·62
Al	3082·2	
		9·30
Cu	3247·5	
		>20
Li[c]	4603·0	

a Si 2528·5 Å preferable but only 3·40 mm. from B 2497·7
b Mn 2576·1 preferable but only 2·45 mm. from P 2553·3
c Li 3232·6 preferable but only 0·76 mm. from Cu 3247·5

Table II

Coefficients of variation obtained from ten replicate determinations of elements in solution, using the Hilger Medium Direct Reader with porous-cup spark excitation, and lithium as the internal standard element

	Solution content, p.p.m.	Coefficient of variation, %
Cu	1·2	±1·10
Al	11·0	±4·35
Mn	4·0	±1·64
Mg	4·8	±3·09
P	125·0	±2·60
Zn	60·0	±7·01

Table III

% errors in the apparent contents found in a solution containing copper 1, manganese 10 and zinc 10 p.p.m. in presence of varying amounts of calcium or potassium

(determinations made with the Hilger Medium Direct Reader and porous-cup spark excitation with chromium and lithium as internal standard elements)

Calcium present, p.p.m.	Zinc		Manganese		Copper	
	Li	Cr	Li	Cr	Li	Cr
0	0·0	0·0	0·0	0·0	0·0	0·0
800	+3·2	+40	−13·4	+3·1	0·0	+18·1
4000	−16·3	+95	−31·6	+7·3	−5·4	+42·0
8000	−25·0	+150	−48·4	+11·6	−17·1	+78·0
Potassium present, p.p.m.						
0	0·0	0·0	0·0	0·0	0·0	0·0
800	−29·4	−5·0	−25·8	+0·8	−13·5	+4·5
4000	−51·5	+2·0	−43·3	−3·1	−50·4	−5·7
8000	−56·7	+34·0	−56·7	−3·1	−59·2	+4·5

and in that of manganese, using chromium. For accurate spectrochemical determinations, the comparison standard solutions and the samples to be analysed should contain similar amounts of extraneous elements.

Analyses of plant ash extracts have not proved feasible by this technique, because after the concentration necessary to obtain adequate sensitivity for the determination of the trace elements, the large and variable amounts of calcium, potassium and other extraneous elements, lead to excessive errors.

Application to soil extracts.—The amounts of copper and manganese extracted from soils by EDTA vary from 0·1 to 20 and from 1 to 400 p.p.m., respectively, and, because of the utility

of EDTA extraction in assessing the copper status of soil, the following method has been developed. Ten g. of air-dry soil are shaken for 1 h. with 50 ml. of 0·05M-EDTA neutralised to pH 7 with ammonia. The suspension is filtered, washed with 50 ml. of distilled water, evaporated to dryness in a small silica basin, and the EDTA ignited off on an electric burner. The ash is evaporated to dryness twice with HCl, taken up in 10 ml. of internal standard solution containing lithium 250 p.p.m., chromium 65 p.p.m. and nitric acid 4% (v/v), and filtered. The apparatus and filter papers are washed with 0·0125M-EDTA solution and distilled water before use, to ensure removal of all traces of copper.

Standard solutions containing copper 0·1–24, manganese 1–400, zinc 1·0–400, lithium 250 and chromium 65 p.p.m., are prepared in a base solution containing calcium 1000, potassium 150 and sodium 100 p.p.m. in 4% (v/v) nitric acid. These solutions are sparked as described above, and the contents of copper and manganese in the samples evaluated from standard curves.

Table IV shows the amounts of zinc, manganese and copper extracted by 2·5% acetic acid from horizons of a soil profile developed on an ultra-basic parent material. Such results are obtained by shaking 20 g. of soil overnight with 800 ml. of 2·5% acetic acid, and setting aside sufficiently long for a clear 500-ml. aliquot to be siphoned off. This is a slow process, but eliminates the danger of contamination from the large filter papers which would otherwise be required. The aliquot is evaporated to dryness, and, after being ashed, taken up in 10 ml. of the internal standard solution. The results are evaluated from standard curves prepared as described above.

Table IV

Amounts (p.p.m.) of zinc, manganese and copper, extracted by 2·5% acetic acid from a soil profile
(results obtained by porous-cup spark excitation with the Hilger Medium Direct Reader)

Depth in in.	Zinc, p.p.m.	Manganese, p.p.m.	Copper, p.p.m.
2–7	6·8	5·6	0·06
8–12	4·0	85	0·19
18–22	2·2	116	0·30
26–30	1·8	115	0·26
38–43	1·2	126	0·25

Normally, amounts varying from 1 to 50 p.p.m. of manganese are extracted by ammonium acetate solution from Scottish soils. This can be determined by evaporating to dryness 250 ml. of neutral normal ammonium acetate extract, derived from 5 g. of soil; the residue obtained on ashing is dissolved in 5 ml. of the internal standard solution as described above.

Manganese determined in ammonium acetate extracts of soils by the direct photometric method and by a permanganate colorimetric procedure, gave satisfactory mean values of 9·7 and 9·9 p.p.m., respectively.

Pelleted rotating disk technique for the analysis of plant materials

A method for the direct determination of such trace elements as zinc, boron, iron, manganese and copper in plant ash, is attractive, and preliminary tests indicated that this might be achieved by using a pressed disk containing plant ash pelleted with a graphite–buffer mixture. In the tentative method described below, the Hilger Medium Direct Reader is employed, and a large amount of calcium and potassium is added to overcome the effects of variations of these elements in the ash.

Two hundred mg. of oven-dry plant material are ashed in a muffle at 450°. The ash, or 20 mg. if the weight of ash is greater than this, is diluted to 50 mg. with the spectroscopic buffer (K_2SO_4 1 part and $CaSO_4$ 1 part). This is ground with 550 mg. of a mixture consisting of boron-free graphite powder 350 mg., buffer 100 mg., cellulose powder 100 mg., Li 1300 p.p.m. as Li_2CO_3 and Cr 500 p.p.m. as $K_2Cr_2O_7$. This powder is compressed with a hydraulic press with a load of 8 tons, into a disk $\frac{1}{2}$ in. in diameter and $\frac{1}{8}$ in. thick. The hardened steel die in which the disk is compressed was constructed so that it forms a conical depression, 4 mm. in diameter and 90° included angle, in the centre of one side of the disk. The counter electrode is a $\frac{1}{8}$-in. diameter boron-free carbon rod with a 70° included angle sharp point. A spark gap of 1·5 mm. is used.

Fig. 1 is a schematic drawing showing the method employed to hold and rotate the disk vertically in the optical axis. The inner back plate of the Hilger FS32 discharge stand has been replaced by a Perspex plate A which holds a nylon bush B, carrying the spindle C. This is rotated at 5 r.p.m. by a synchronous motor through a flexible polythene shaft D. The disk is held against eight driving teeth on the end of spindle C, by the spring-loaded rod E whose hemispherical end enters the central depression of the disk. Current from the source is carried to the disk by spring F and rod E. The discharge is stabilised by four air jets G, with an air flow of 6 l./min. When the stand is used for porous cup excitation, the nylon bush B is unscrewed from plate A, and replaced by a nylon plug, while the whole spring-loaded rod movement, E, F and G, is withdrawn from the nylon-covered stainless steel bush H, and replaced by a second spring-loaded electrode clamp similar to J, which carries the counter electrode.

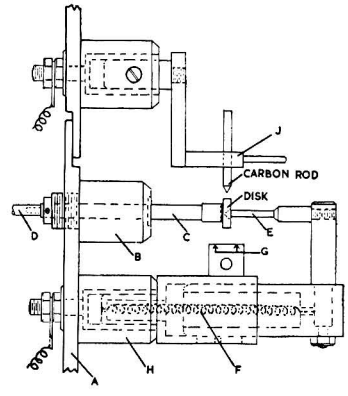

FIG. 1.—*Schematic drawing of the spring-loaded rotating electrode holder employed for the analyses of plant materials with the Hilger Medium Direct Reader*

A Perspex plate B Nylon bush
C Rotating spindle
D Flexible polythene shaft
E Hemispherical-ended rod
F Spring G Air jets
H Nylon-covered stainless steel bush
J Spring-loaded counter electrode clamp

The disks are excited by a triggered low-voltage a.c. condenser discharge of 13·4 μF capacity, 0·13 mH inductance and 9 ohms resistance, using a pre-spark of 12 sec. and exposure time of 1 min. 40 sec.

The standard samples used for the preparation of the standard curves are synthetic plant ashes prepared in a base similar to that described by Farmer[5] and contain 10–1000 p.p.m. of zinc, boron and copper, and 100–10,000 p.p.m. of iron and manganese. Chemically analysed plant materials are employed to prepare the standard curves for the determination of silicon and aluminium.

Chromium has proved a better internal standard than lithium for the determination of all the elements by this method, the coefficients of variation derived from replicate analyses of a plant ash being about half those found using lithium. Table V gives a comparison of the coefficients of variation resulting from replicate determinations of the elements in a pasture herbage using chromium as the internal standard and employing varying source parameters. Source parameters A, now used, give the most satisfactory coefficients of variation, the poor reproducibility of iron being the result of variable contamination from the steel die employed in the preparation of the disk.

Table V

Coefficients of variation from replicate determinations of elements in a pasture herbage by the rotating disk method using various source parameters

(Hilger Medium Direct Reader was employed with chromium as the internal standard element)

Source parameters	Coefficients of variation, %						
	Zinc, 45 p.p.m.	Boron, 24 p.p.m.	Phosphorus, 0·28 %	Iron, 122 p.p.m.	Magnesium, 0·23 %	Manganese, 111 p.p.m.	Copper 10.0 p.p.m.
A	±1·9	±2·8	±3·2	±17·9	±3·2	±3·3	±5·4
B	±3·5	±3·8	±6·2	±28·0	±4·9	±2·9	±7·4
C	±3·1	±2·1	±1·3	±37·0	±15·5	±3·4	±8·2

A a.c. arc: 13·4 μF, 0·13 mH, 9 ohms
B ,, ,, 20 μF, 0·13 mH, 9 ohms
C Uni-directional pulsed arc: 40 μF, 0·03 mH, 1 ohm

A comparison is given in Table VI of the results obtained for boron in plant materials by the rotating disk method and at Rothamsted Experimental Station by a dianthrimide colorimetric procedure, the agreement for most types of plant materials being satisfactory. The

method also appears suitable, at its present stage of development, for the determination of zinc, manganese and copper. The determination of silicon, magnesium, aluminium and phosphorus is being investigated.

Table VI

Comparison of results obtained for boron in plant materials by the rotating disk method with the Hilger Medium Direct Reader, and by dianthrimide colorimetric procedure

	Boron found, p.p.m.			Boron found, p.p.m.	
	Rotating disk method	Colorimetric procedure		Rotating disk method	Colorimetric procedure
Apple leaves	28	28	Lucerne	31	33
Pasture herbage	10	10	Oat grain	4·4	1·5
Wheat grain	1·4	2	Tomato leaves	47	52
Wheat straw	4·9	4	Pasture herbage	12	12
Broccoli	37	39	Kale leaves	27	28
Cauliflower	27	29	Cabbage leaves	22	21

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MYCOLOGICAL PRODUCTION OF CITRIC AND OXALIC ACIDS FROM CANE MOLASSES. I.—Effects of some Cultural Conditions and Supplements of Ferrocyanide and Phosphate

By IBRAHIM R. SHIMI and MOUSTAFA S. NOUR EL DEIN

Studies are made of the effects of the following factors influencing the biosynthesis of citric and oxalic acids from cane molasses in surface cultures of some strains of *Aspergillus niger*, (i) cultural conditions, (ii) presence of ferrocyanide and phosphate supplements either separately or together. The organic acids biosynthesised under such conditions were characterised by one-dimensional paper chromatography. The results are discussed in relation to the findings of previous workers and the metabolic activities of the organisms.

Introduction

Literature concerning the mycological production of citric acid from cane molasses is rather scanty. Perlman *et al.*[1] studied the mycological synthesis of the acid from cane molasses using

a strain of *Aspergillus niger*. They attributed the low yields of the acid obtained to the inorganic materials of the molasses. Since oxalate is always present in the metabolism solutions of different strains of *A. niger*, several workers have devoted their efforts to explore the factors which affect the biosynthesis of this acid. Currie & Thom[2] demonstrated that by controlling the pH and the concentration of inorganic salts, the ratio of citric/oxalic acid could be varied considerably. Other workers[3–5] have all studied the extent to which the formation of citric and oxalic acids is influenced by the initial pH values of the synthetic media which they used.

Potassium ferrocyanide has been used to induce metal deficiency in molasses, and several authors (e.g., [5–9]) have found that the yields of citric acid in cultures of *A. niger* were improved considerably thereby.

Quilico & Dicapua[10] using untreated beet molasses found that optimum yields of citric acid (50–55% of available sugar) were obtained when 0·01–0·02% of monopotassium phosphate was added to the medium. Kovats[11] employing untreated beet molasses, but probably using another strain of *A. niger*, found that the phosphate supplement lowered the yields of citric acid. Shu & Johnson[12] reported that the most rapid production of citric acid in submerged cultures of *A. niger* requires a concentration of phosphate in excess of the optimum value required for growth. Steel *et al.*[13] found that the addition of phosphate supplement was undesirable for citric acid production from Chatham molasses. Martin & Steel[14] were able to trace the formation of organic acids in cultures of *A. niger* grown on molasses medium.

In view of these various conclusions, it was decided to explore the activities of some strains of *A. niger* when cultivated on Abou Korkas blackstrap cane molasses.

Experimental

(A) *Effects of some cultural conditions*

The moulds employed were the following strains of *A. niger*: B_8, B_{7a}, C_{11e}, C_{10a} and NI which were kindly supplied by Prof. T. K. Walker, and strain 609 which was generously offered by the North Regional Research Laboratories, Peoria (U.S.A.). These strains were subcultured every 10 days for 20 successive times on molasses/ammonium nitrate/agar slants so that the organisms were adapted to this molasses. The composition of such solid medium is (g./100 ml.) molasses sugar 15·0; ammonium nitrate 0·2 and agar 2·0.

The molasses used was of the blackstrap type of the Abou Korkas Refining Co. The analytical data for this molasses are (g./100 ml.): Mg, 0·34; K, 4·03; Na, 0·58; Ca, 1·3; P, 0·061; N, 0·4; Fe^{2+}, 0·23 and reducing sugars 54·4%. Lactic and aconitic acids determined by the methods of Friedman & Grasser[15] and Aubler & Robert,[16] respectively, were 0·63% and 5·9% (w/v).

The changes in the free acidity of the solutions represent the difference in volume of 0·1N-NaOH solution required to neutralise the preparations before and after incubation for a definite period at 28°. Oxalic acid was precipitated as the calcium salt from acid solution. The calcium oxalate was then dissolved in 0·01N-H_2SO_4 and titrated against standard permanganate. Adjustment of the initial pH values of the molasses solutions by either HCl or NaOH was conducted aseptically after sterilisation. The other experimental methods were as described by Shimi.[17]

(1) *Effects of different nitrogen sources*

Six different N sources were added separately as supplements to dilute molasses solutions in such amounts that each ml. of the resulting media contained equal amounts of added nitrogen. The nitrogen compounds added were as follows (g./100 ml.): $NaNO_3$, 0·64; NH_4NO_3, 0·11; $(NH_4)_2SO_4$, 0·46; $(NH_4)_2HPO_4$, 0·49; NH_4Cl, 0·40 and urea, 0·23. Dilute solutions of the molasses without added nitrogen were employed as media for growth of the different strains. The initial pH of all the solutions was adjusted to 6·8. Aliquots of 25 ml. of the solutions were placed in 150-ml. Erlenmeyer flasks, a quintuplicate set of flasks containing a given nitrogen source being used for the growth of each strain. The flasks were incubated for 10 days, sterilised and the different estimations were carried out. The results are set out in Table I.

Table I

Behaviour of A. niger *in presence of different nitrogen sources*

25-ml. portions of media, initial pH 6·8, final pH 2–7, initial molasses sugar concentrations 14·4%. (Results and calculations based on 125 ml. of media)

	Strain of *A. niger*	—	Urea	Sodium nitrate	Ammonium sulphate	Ammonium phosphate	Ammonium chloride	Ammonium nitrate
Change in titratable acidity, ml. 0·1N	B_8	+83·0	−135·5	+37·2	−105·8	−195·0	−152·6	+213·5
	B_{7a}	−30·0	−195·3	+37·2	−195·3	−134·6	−125·0	+117·5
	NI	+130·5	+158·6	+360·5	+96·5	+287·2	+483·5	+663·2
	C_{10a}	+48·5	−45·0	+62·2	−105·7	−65·4	−50·5	+117·5
	609	+37·2	+75·5	+187·5	−60·0	+45·5	+50·0	+482·6
	C_{11e}	+114·6	−15·7	+187·2	+135·2	+45·0	+25·0	+528·5
Felt weight, g.	B_8	4·365 ±0·0729	4·465 ±0·0149	7·665 ±0·0185	4·735 ±0·0056	6·365 ±0·0432	5·730 ±0·0348	6·310 ±0·0279
	B_{7a}	2·580 ±0·0250	4·910 ±0·0041	4·980 ±0·0174	5·010 ±0·0419	6·160 ±0·0081	5·290 ±0·0442	4·630 ±0·0168
	NI	4·510 +0·030	5·910 +0·0483	5·820 ±0·0144	4·535 ±0·0234	4·760 ±0·0745	5·375 ±0·0290	6·284 ±0·0227
	C_{10a}	3·34 ±0·0353	4·090 ±0·0151	6·255 ±0·0118	4·665 ±0·0167	6·840 ±0·0246	4·960 ±0·0563	5·160 ±0·0133
	609	4·935 ±0·1703	3·795 ±0·0088	5·150 ±0·029	4·800 ±0·0267	4·925 ±0·0262	5·383 ±0·0737	5·630 ±0·0868
	C_{11e}	4·465 ±0·0859	3·830 ±0·0086	5·865 ±0·034	4·760 ±0·0336	5·821 ±0·0336	6·190 ±0·0223	5·430 ±0·0188
Sugar consumed, g.	B_8	10·50	9·66	9·87	12·06	11·85	11·29	12·50
	B_{7a}	7·48	9·26	10·45	11·99	11·77	11·56	12·65
	NI	10·78	9·26	10·50	12·38	11·45	11·62	13·15
	C_{10a}	11·50	9·14	10·23	11·44	11·43	11·71	12·09
	609	9·62	9·19	10·25	11·78	11·27	10·98	12·85
	C_{11e}	10·35	9·45	9·93	11·11	12·26	11·34	13·10

(2) *Effects of various concentrations of molasses sugar*

In these tests ammonium nitrate only was used as the added nitrogen source. The experiment was conducted as described in (1) above. This experiment clearly demonstrates that a molasses sugar concentration of 15% was the most suitable for production of acids, formation of mycelia and uptake of sugar.

(3) *Effects of the initial pH values at different incubation periods*

Eighteen 1-l. Erlenmeyer flasks were set up, each charged with 200 ml. of diluted molasses and supplemented with ammonium nitrate (0·2 g./100 ml.). The flasks were sterilised and the pH values of the contents were adjusted. Each organism was allowed to grow on media of different pH values. Ten-ml. portions of the metabolism solutions were withdrawn aseptically every 24 h. for analysis. Fig. 1 represents the changes which took place in the metabolism solutions of strain B_8. The results for the other strains are in general similar to those for B_8 and are not detailed here.

(B) *Effects of ferrocyanide and phosphate supplements*

The strains of *A. niger* used were NI and C_{11e} and the experimental procedures were as described above. Addition of ferrocyanide was made by the method of Martin & Waters.[8] The pH was initially adjusted in all cases at 5·5.

(1) *Effects of monopotassium phosphate*

Twelve 1-l. Erlenmeyer flasks were set up, each charged with 200 ml. of a solution of 15% molasses sugar solution containing 0·2 g.-% of ammonium nitrate. Five different concentrations of the monopotassium phosphate were used, viz., (g./100 ml.): 0·001, 0·005, 0·010, 0·025 and 0·05. Control flasks, without added phosphate, were also included. The results are set out in Table II.

(2) *Effects of optimum phosphate together with various levels of ferrocyanide*

A further six 1-l. Erlenmeyer flasks were set up, as in B(1) and the contents sterilised. The

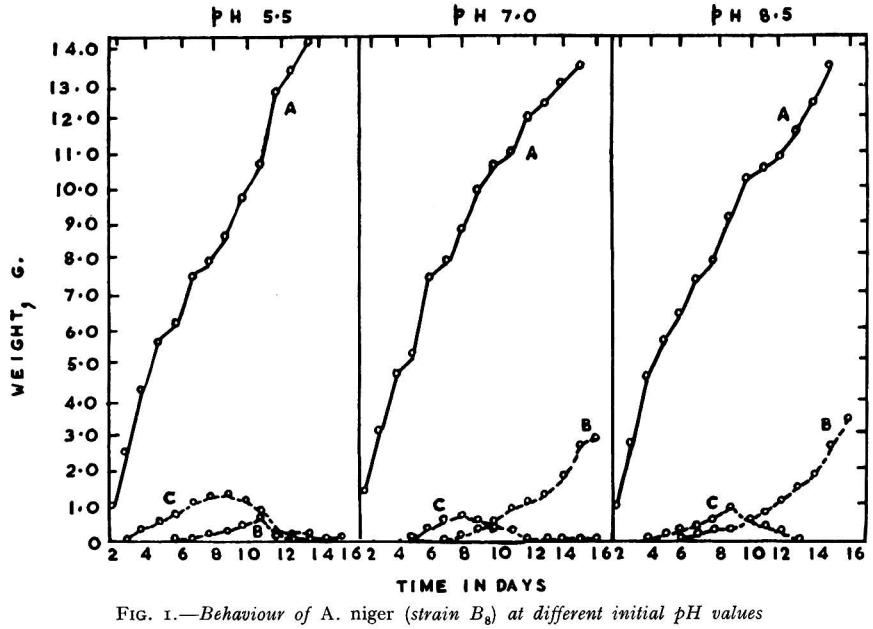

FIG. 1.—*Behaviour of* A. niger (*strain* B_8) *at different initial pH values*

A = Sugar consumed
B = Oxalic acid produced
C = Citric acid produced

Table II

Behaviour of A. niger *in presence of monopotassium phosphate*
Initial concentration of molasses sugars 14·92 g./100 ml. of medium

Initial pH 5·5 Final pH 2·0–4·0
(Results and calculations based on 100 ml. of media)

Strain of *A. niger*	Potassium phosphate, g./100 ml.	Sugar consumed, g.			Citric acid, g.			Oxalic acid, g.		
		Incubation period in days								
		9	12	15	9	12	15	9	12	15
C_{11e}	0·00	9·18	12·35	14·92	3·78	3·32	3·07	0·42	0·79	0·95
	0·001	9·66	12·64	14·92	3·17	4·16	3·35	0·38	0·73	0·86
	0·005	9·42	12·75	14·92	4·33	5·25	4·73	0·27	0·66	0·73
	0·010	9·83	13·42	14·92	6·18	7·52	7·01	0·19	0·62	0·75
	0·025	10·24	13·80	14·92	5·67	6·27	5·78	0·88	0·52	0·68
	0·050	13·37	14·12	14·92	2·18	2·76	2·00	0·03	0·32	0·42
NI	0·00	10·79	13·17	14·92	4·30	4·81	1·47	0·75	0·84	0·93
	0·001	10·60	12·82	14·92	4·50	5·07	3·36	0·04	0·06	0·07
	0·005	10·45	13·07	14·92	4·61	5·34	4·12	0·04	0·05	0·07
	0·010	11·43	14·52	14·92	5·27	6·37	4·47	0·02	0·04	0·05
	0·025	10·70	13·66	14·92	4·00	4·88	2·55	0·01	0·02	0·03
	0·050	10·09	12·76	14·92	1·25	2·12	1·58	0·00	0·00	0·00

following amounts of ferrocyanide, $K_4Fe(CN)_6,3H_2O$, were added (g./ml.): 0·12, 0·14, 0·16 and 0·18. The liquids were left to cool overnight and subsequently potassium phosphate (0·01 g./100 ml.) was added to each flask. Control flasks without ferrocyanide but with phosphate were also set up. The results are recorded in Table III.

(3) *Effects of the phosphate together with optimum ferrocyanide*

The procedure of (2) above was used except that different concentrations of phosphate were added. The phosphate was supplied in the following amounts (g./100 ml.): 0·005, 0·010 and 0·025, the concentrations of ferrocyanide being 0·14 for C_{11e} and 0·16 for NI. The results are set out in Table IV.

Table III

Behaviour of A. niger *in presence of different concentrations of ferrocyanide with optimum phosphate*

Initial concentration of molasses sugar 15·36 g./100 ml. of medium
0·01 g. of KH_2PO_4 per 100 ml. of medium

Initial pH 5·5 Final pH value 1·5–3·0

(Results and calculations based on 100 ml. of media)

Strain	Ferrocyanide in medium, g./100 ml.	Sugar consumed, g.			Citric acid, g.			Oxalic acid, g.		
		Incubation period in days								
		9	12	15	9	12	15	9	12	15
C_{11e}	(Control)	9·40	12·72	13·75	5·93	7·98	6·65	0·150	0·485	0·605
	0·14	12·59	13·88	15·36	5·22	8·05	6·17	0·073	0·120	0·197
	0·16	12·16	13·52	15·36	7·45	10·85	7·57	0·014	0·040	0·078
	0·18	12·64	14·05	15·36	2·72	5·82	4·85	0·006	0·015	0·033
NI	(Control)	11·13	13·16	15·36	4·87	6·67	4·82	0·025	0·038	0·062
	0·12	12·00	13·88	15·36	4·62	7·18	5·32	0·027	0·034	0·058
	0·14	10·89	13·98	15·36	6·88	9·98	7·30	0·019	0·030	0·056
	0·16	12·19	14·05	15·36	3·75	5·55	4·09	0·012	0·023	0·039

Table IV

Behaviour of A. niger *in presence of monopotassium phosphate with optimum ferrocyanide*

Initial concentration of molasses sugar 15·36 g./100 ml.

Ferrocyanide (g./100 ml.): C_{11e} 0·16, NI 0·14

Initial pH 5·5 Final pH 1·5–3·6

(Results and calculations based on 100 ml. of media)

Strain	Composition of media, g./100 ml.	Sugar consumed, g.			Citric acid, g.			Oxalic acid, g.		
		Incubation period in days								
		9	12	15	9	12	15	9	12	15
C_{11e}	(Control)	12·11	14·88	15·36	6·37	8·25	6·62	0·056	0·110	0·223
	0·005	12·57	14·25	15·36	5·84	7·74	6·00	0·035	0·056	0·093
	0·010	11·68	13·82	15·36	6·75	10·80	7·35	0·035	0·037	0·072
	0·025	11·88	12·85	15·36	3·75	4·66	3·16	0·010	0·033	0·052
NI	(Control)	11·13	14·62	15·36	7·85	8·45	5·88	0·092	0·154	0·216
	0·005	11·58	15·36	15·36	5·54	7·56	4·05	0·035	0·118	0·114
	0·010	10·59	13·72	15·36	6·58	9·82	7·26	0·017	0·030	0·050
	0·025	10·52	13·62	15·36	4·27	5·75	3·69	0·003	0·028	0·038

(4) *Identification of the organic acids obtained in presence of phosphate and/or ferrocyanide*

Since the addition of the two salts has altered considerably the citric/oxalic ratios, it is not unreasonable to assume that some changes in the metabolic activities of the organisms had occurred. Thus, an attempt was made to identify the organic acids in the metabolism solutions after different incubation periods by one-dimensional paper chromatography. Each of the two organisms was grown separately on 200-ml. portions of the following media (g./100 ml.):

(*a*) Purely synthetic medium: NH_4NO_3, 0·2; KH_2PO_4, 0·03; $MgSO_4,7H_2O$, 0·025 and sucrose, 15·0 (Table V).

(*b*) Molasses medium containing: NH_4NO_3, 0·2 and 15 of molasses sugar (Table VI).

(*c*) Molasses medium of the same composition as in (*b*) with addition of ferrocyanide, 0·14 g. for strain NI and 0·16 g. for strain C_{11e} (Table VII).

(*d*) Composition of the medium as in (*c*) with addition of 0·05 of potassium monohydrogen phosphate (Table VIII).

Ten-ml. portions were withdrawn every 24 h. for chromatographic analysis and quantitative determination of citric and oxalic acids. Whatman No. 1 filter paper was spotted with the different solutions in the centre of the top edge of the paper and spots of the solutions of authentic acids were located on both sides of them. The spots were developed by spraying the chromatograms with an alcoholic solution of bromophenol blue (400 mg./1 l.) in 96% alcohol adjusted to pH 5·5–6.

Confirmatory tests were made to establish the nature of the acids detected.

Table V

Formation of organic acids by A. niger *grown on synthetic media*

Acids	Incubation period in days									
Strain C_{11e}	1	2	3	4	5	6	7	8	9	10
Citric (g.)	0·26	0·46	0·64	1·00	1·80	2·48	3·82	4·65	5·20	5·700
Oxalic (g.)	0	0	0·068	0·105	0·134	0·155	0·172	0·265	0·47	0·634
Gluconic	++	++	++	++	++	++	++	++	++	++
Malic	0	0	0	0	0	0	0	0	+	+
Fumaric	0	0	0	0	0	0	0	0	0	0
α-Ketoglutaric	0	0	0	0	0	0	0	0	0	0
Succinic	0	0	0	0	0	0	0	0	0	0
Pyruvic	0	0	0	0	0	0	0	0	0	0
Lactic	0	0	0	0	0	0	0	0	0	0
Aconitic	0	0	0	0	0	0	0	0	0	0
Strain NI										
Citric (g.)	0·15	0·66	1·15	1·60	2·45	3·50	4·75	5·33 *G*	6·85	7·50
Oxalic (g.)	0	0·074	0·125	0·266	0·320	0·418	0·520	0·783	0·883	1·035
Gluconic	+	+	+	+	++	++	++	++	++	++
Malic	0	0	0	0	0	0	0	0	0	+
Fumaric	0	0	0	0	0	0	0	0	0	0
α-Ketoglutaric	0	0	0	0	0	0	0	0	0	0
Succinic	0	0	0	0	0	0	0	0	0	0
Pyruvic	0	0	0	0	0	0	0	+	+	+
Lactic	0	0	0	0	0	0	0	0	0	0
Aconitic	0	0	0	0	0	0	0	0	0	0

0	Undetected	
+	Trace	(0·1–0·2 g.-%)
++	Low	(0·2–0·5 g.-%)

Table VI

Formation of organic acids by A. niger *grown on molasses medium*

Acids	Incubation period in days									
Strain C_{11e}	1	2	3	4	5	6	7	8	9	10
Citric (g.)	0	0·15	0·25	0·43	0·70	1·00	1·48	2·72	3·45	2·56
Oxalic (g.)	0	0	0	0·066	0·078	0·095	0·113	0·340	0·656	1·205
Gluconic	++	++	++	++	++	++	++	+++	+++	+++
Malic	0	+	+	+	+	+	+	++	++	++
Fumaric	0	0	0	0	0	0	0	0	0	0
α-Ketoglutaric	0	0	+	+	+	+	+	+	+	+
Succinic	0	0	0	0	0	0	0	0	0	0
Pyruvic	0	0	0	0	0	0	0	0	0	0
Lactic*	++	++	++	++	++	++	++	++	++	++
Aconitic*	++	++	++	++	++	++	++	+	+	+
Strain NI										
Citric (g.)	0·08	0·13	0·20	0·56	1·50	1·62 *G*	2·25	2·48	3·40	4·85
Oxalic (g)	0	0	0	0	0·106	0·365	0·760	0·825	0·944	1·460
Gluconic	+	++	++	++	++	++	++	+++	+++	+++
Malic	0	0	0	0	0	+	+	+	+	+
Fumaric	0	0	0	0	+	+	+	+	+	+
α-Ketoglutaric	0	+	+	+	+	+	+	+	+	+
Succinic	0	0	0	0	0	0	0	0	+	+
Pyruvic	0	+	+	+	+	+	+	+	+	+
Lactic*	+++	+++	+++	++	+	0	0	0	0	0
Aconitic*	+++	+++	+++	++	+	+	+	0	0	0

0	Undetected	
+	Trace	(0·1–0·2 g.-%)
++	Low	(0·2–0·5 g.-%)
+++	Medium	(0·5–1·5 g.-%)
++++	High	(Above 1·5 g.-%)

* present 0·5–1·5 g.-% in original molasses: all other acids not detected

The estimation of each acid was effected by employing two chromatograms, one of which was sprayed with the dye solution and the R_F of the spots determined. The other was cut into three longitudinal strips, one of which contained the spots from the unknown and the others the spots from the authentic acids. The strips were sprayed with the dye solution and the zone of the middle strip lying between every two spots from any of the authentic acids were

Table VII

Effect of ferrocyanide on the production of organic acids from cane sugar molasses by A. niger

Acids	Incubation period in days									
	1	2	3	4	5	6	7	8	9	10
Strain C_{11e}										
Citric (g.)	0·33	0·68	1·25	1·85	2·77	3·32	5·08	6·75	8·20	9·25
Oxalic (g.)	0	0	0·005	0·011	0·032	0·045	0·117	0·265	0·310	0·483
Gluconic	+	+	++	++	++	++	++	+++	+++	+++
Malic	0	+	+	+	+	+	+	++	++	++
Fumaric	0	0	0	0	0	0	0	0	0	0
α-Keto-glutaric	0	0	+	+	+	+	+	+	+	+
Succinic	0	0	0	0	0	0	0	0	0	0
Pyruvic	0	0	0	0	0	0	0	0	0	0
Lactic*	++	++	++	++	++	++	++	++	++	++
Aconitic*	++	++	++	++	++	++	++	++	++	++
Strain NI										
Citric (g.)	0·23	0·78	1·65	2·27	3·20	4·15	5·80	7·76	8·95	9·26
Oxalic (g.)	0	0	0·010	0·116	0·214	0·366	0·582	0·770	1·083	1·164
Gluconic	+	++	++	++	+++	+++	+++	++++	++++	+++
Malic	0	0	0	0	0	+	+	+	+	+
Fumaric	0	0	0	0	0	+	+	+	+	+
α-Keto-glutaric	0	+	+	+	+	+	+	+	+	+
Succinic	0	0	0	0	0	+	+	+	+	+
Pyruvic	0	+	+	+	+	+	+	+	+	+
Lactic*	+++	+++	+++	+++	+	0	0	0	0	0
Aconitic*	+++	+++	+++	+	+	+	+	+	+	0
Unknown (R_F 0·23)	0	+	+	+	+	+	+	+	+	0

Symbols as in Table VI

* present 0·5–1·5 g.-% in original molasses: all other acids not detected

Table VIII

Effects of ferrocyanide and phosphate on the production of organic acids from cane sugar molasses by A. niger

Acids	Incubation period in days									
	1	2	3	4	5	6	7	8	9	10
Strain C_{11e}										
Citric (g.)	0·25	0·77	0·82	3·65	3·80	4·67	5·83	6·50	7·52	9·75
Oxalic (g.)	0	0	0	0	0	0·007	0·011	0·014	0·017	0·038
Gluconic	+	+	+	+	++	++	+++	+++	++++	++++
Malic	+	+	+	+	+	+	+	++	++	++
Fumaric	0	0	0	0	0	0	0	0	0	0
α-Keto-glutaric	0	0	+	+	+	+	+	+	+	+
Succinic	0	0	0	0	0	0	0	0	0	0
Pyruvic	0	0	0	0	0	0	0	0	0	0
Lactic*	++	++	++	++	++	++	++	++	++	++
Aconitic*	++	++	++	++	++	++	++	++	++	++
Strain NI										
Citric (g.)	0·12	0·55	1·20	1·85	2·56	3·46	3·72	4·40	6·67	8·70
Oxalic (g.)	0	0	0	0	0	0	0·010	0·013	0·020	0·043
Gluconic	0	+	++	+++	+++	+++	+++	+++	++++	++++
Malic	0	0	0	0	0	+	+	+	+	++
Fumaric	0	0	0	0	0	+	+	+	+	+
α-Keto-glutaric	0	+	+	+	+	+	+	+	+	+
Succinic	0	0	0	0	0	0	0	0	+	+
Pyruvic	0	+	+	+	+	+	+	+	+	+
Lactic*	+++	+++	+++	++	+	+	0	0	0	0
Aconitic*	+++	+++	+++	++	+	+	+	+	+	0
Unknown (R_F 0·23)	0	+	+	+	+	+	+	+	+	0

Symbols as in Table VI

* present 0·5–1·5 g.-% in original molasses: all other acids not detected

cut off and eluted with the minimum amount of water. Confirmatory tests were applied to the elutes after being concentrated to a few drops. Gluconic, malic and succinic acids were estimated as described by Eegriwe[18] and Feigl,[19] respectively. The zones of the middle strip of the chromatograms which were suspected to contain either α-ketoglutaric or pyruvic acids were sprayed with a freshly prepared 0·05% solution of *o*-phenylenediamine in 10% aqueous trichloroacetic acid and then dried at 100° for 2 min. The spots fluoresced with a yellow-green colour at u.v. light. The two keto-acids were also precipitated from the metabolism solutions as their 2,4-dinitrophenylhydrazones and then identified chromatographically.

Discussion

The natural nitrogen sources which already exist in the molasses were unsuitable for production of appreciably high changes in the titrable acidity. Of the other nitrogen sources employed, ammonium nitrate was the best for the production of acids, and the optimum concentration of molasses sugars was 15%. In agreement with the findings of Currie & Thom[2] and Gerhardt *et al.*[5] the accumulation of citric acid in cultures of the moulds used was stimulated at initial acidic pH values but that of oxalic acid was retarded : the reverse was found at alkaline pH values. A possible reason for this is that the utilisation of oxalic acid is stimulated by the drop in the pH value, while that of citric acid is restricted. Worthy of mention is that the response of the biosynthesis of citric and oxalic in molasses cultures to changes in the initial pH values is similar to that described by workers who employed synthetic media. Thus the complex nature of molasses did not induce radical changes in such metabolic processes under the cultural conditions applied.

Increasing concentrations of phosphate up to 0·1% increased the yields of citric acid and suppressed the formation of oxalic acid. The suppressing influence of ferrocyanide[10] and phosphate on oxalic acid formation was increased when the two salts were present together. On the other hand the presence of the two salts together had a synergistic stimulatory effect on the formation of citric acid.

Tracing of the bio-synthesised organic acids provides evidence that the metabolic activities of the two moulds were disturbed by the complex nature of molasses in such a fashion that some intermediates of the tricarboxylic acid cycle, e.g., malic, fumaric, α-ketoglutaric, pyruvic and succinic acids, accumulated in the metabolism solutions. The yields of citric and oxalic acids were reduced while that of gluconic acid was stimulated. Thus the nature of the molasses most probably influences the different stages of the tricarboxylic cycle in such a fashion that some metabolic changes are stimulated (presumably from citric acid down to α-ketoglutaric acid via isocitric acid) while others (from α-ketoglutaric acid to malic) are retarded. Thus the accumulation of α-ketoglutaric, succinic, fumaric and malic acids would be anticipated. This was actually the case in the molasses cultures but not in cultures of synthetic medium. In the latter types of cultures, higher yields of citric and oxalic acids were detected but no intermediates could be detected, except traces of malic and pyruvic acids which appeared after a comparatively long period of incubation.

Except for its promoting effect on the accumulation of citric acid and its restricting influence on the formation of oxalic acid, ferrocyanide induced no other detectable differences. Martin[20] attributed the exceptionally high yields of citric acid in presence of the ferrocyanide to the poisoning effect of this salt on the activities of the isocitric dehydrogenase. If this actually occurs, aconitic acid as well as isocitric would tend to accumulate, but these two acids could not be detected experimentally. Also Martin's conclusion does not agree with the findings that the utilisation of aconitic acid of molasses was slightly restricted by ferrocyanide. The above results can be reasonably explained by assuming that the ferrocyanide not only affects the isocitric dehydrogenase but also inhibits the *cis*-aconitase. Dickman & Cloutier[21] found that *cis*-aconitase was poisoned by cyanide which is known to be produced from the ferrocyanide in acid solution. Ferrocyanide may also affect the aconitase enzyme by depriving the metabolic pool from enough supply of Fe^{2+} which is known to be a co-factor for this enzyme.[22]

The above assumption would agree with the observed effect of ferrocyanide on the formation of oxalic acid. If oxalic acid is in part produced by the oxidation of glyoxylic acid which would in turn be formed from isocitric acid through the activity of isocitricase,[23] the observed effect

of ferrocyanide would be anticipated. In such a case ferrocyanide would restrict the transformation of citric acid to isocitric acid which might consequently be transformed to glycollic acid and finally to oxalic acid.

That oxalic acid was still formed in presence of comparatively high concentrations of ferrocyanide may be attributed to other routes, insensitive to the inhibitor, by which oxalic acid was still synthesised.

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DRYING OF SEAWEEDS AND OTHER PLANTS. II.*—Through-circulation Drying of *Laminaria longicruris*†

By J. H. MERRITT

Tests on the through-circulation drying of *Laminaria longicruris* on a semi-commercial scale in a batch dryer have been made to determine the optimum conditions. There was a maximum feasible loading of approximately 5 lb./cu. ft. for the dryer. The effects of variations in the rate of air flow, of amount of air recirculated, of air-flow reversal and of temperature (160–220 °F) have been studied with particular reference to output and efficiency.

Introduction

In a previous investigation the optimum conditions for the drying of the rockweed, *Ascophyllum nodosum*, were determined on a semi-commercial scale in a batch dryer.[1] The work has now been extended in a similar manner to the drying of a common, local kelp,

* Part I: *J. Sci. Fd Agric.*, 1958, **9**, 300
† Issued as N.R.C. No. 5960

Laminaria longicruris. Gardner & Mitchell[2] have investigated the drying characteristics of three species of *Laminaria* in a through-circulation dryer on a laboratory scale and Reid & Jackson[3] have studied the non-thermal drying of several species of seaweed. Few observations have thus been made on the drying of kelp which is the main source for the commercial preparation of alginates.

Experimental

Dryer

The dryer used has been described in detail previously.[1] The drying chamber was 2 ft. wide × 4 ft. deep × 4 ft. high. The air was heated by steam coils and circulated by a centrifugal fan.

Seaweed

The seaweed was obtained locally in 500-lb. lots and stored in burlap bags. Tests were conducted from July to December, 1957, on plants which were harvested 1–2 days before drying. The plants received no preliminary treatment. Earlier drying was impossible because of the location of the beds.

Procedure

The whole plant was hung parallel to the direction of air flow from rods 2 ft. long which spanned the top of the drying chamber. The plants were looped over the rods two or three times according to their length so that they hung a distance of nearly 4 ft.

The initial moisture content as determined by an infra-red moisture balance was usually very close to 83% and evaporation was continued until the final moisture content was less than 15%. For comparative purposes, final moisture contents of 15%* were used with the corresponding drying times arrived at by interpolation. No sampling was done during runs but otherwise the procedure was similar to that previously employed for *Ascophyllum nodosum*.[1]

Results

Independent variables

Loading and air mass flow were considered to be largely independent of other variables.

Preliminary tests showed that through-circulation drying of whole and minced weed was uneven because of poor circulation of air through the bed. Satisfactory drying of whole plants was obtained by loading as described above. Output was found to decrease slightly and drying was uneven when loadings exceeded 150 lb. of wet weed. The best loading was at 150 lb. on ten rods spaced nearly 5 in. apart.

With a loading of 150 lb. of wet weed or 3·2 lb. of bone-dry solid (B.D.S.)/sq. ft., output was measured for various air velocities at zero recirculation. The results are shown in Fig. 1. Within the range studied, output was nearly directly proportional to the air mass flow up to 20 lb. dry air/min./sq. ft.

Differences in final moisture content were found from top to bottom, with a maximum where the weed came in contact with the rods. These differences were greatly reduced by one reversal of air flow about half-way through the run.

The frond dried more rapidly than the stipe. The final moisture content was the average for the batch. The stipes accounted for about one-tenth of the total weight. At a moisture content of 15% the plant becomes very brittle and may be pulverised readily in a hammer mill.

Rate of drying

Values of output are based on the total drying time for each batch. It was found that after about 5 min., during which time adiabatic conditions were reached, there was a fall in the drying rate throughout the rest of the test. The maximum drying rate was approximately double the average for the run.

* C.D.S. = commercial dry solid, 15% water

Static pressure drop

The static pressure drop was measured for a loading of 3·2 lb. B.D.S./sq. ft. The result is shown in Fig. 2. These values are for a dry batch of kelp and are approximately 10% lower than for the corresponding wet batch.

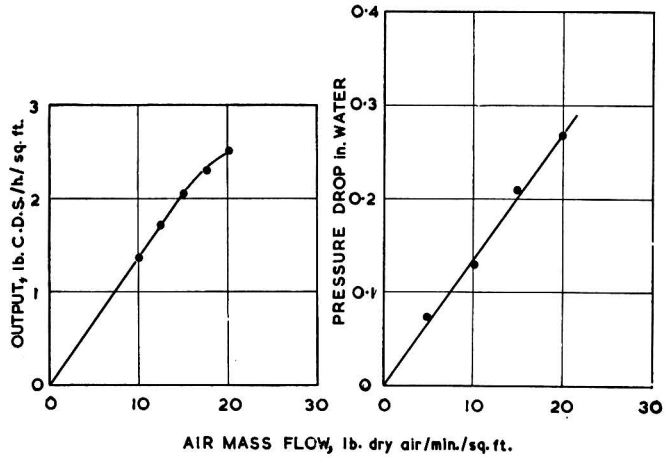

FIG. 1.—*Output vs. air mass flow*
Loading 3·2 lb. B.D.S./sq. ft.
Recirculation zero
Control temperature 200° F

FIG. 2.—*Static pressure drop*
Loading 3·2 lb. B.D.S./sq. ft.
Control temperature 200° F

Temperature and recirculation

With a loading of 3·2 lb. B.D.S./sq. ft. on ten rods and an air mass flow of 20 lb. of dry air/min./sq. ft., tests were made at various control temperatures and recirculation values. The results are shown in Fig. 3.

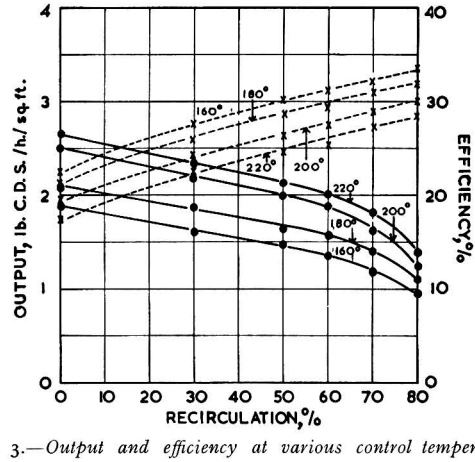

FIG. 3.—*Output and efficiency at various control temperatures*
Air mass flow 20 lb. of dry air/min./sq. ft.
Loading 3·2 lb. of B.D.S./sq. ft.
Make-up air temperature 70° F
Output ——·—— efficiency - - - - × - - - -

With the above conditions, and with the ideal dryer chart as a guide, runs of varying recirculation[1] were made. The results are shown in Table I for runs with ideal efficiency at 40–50% based on the definition of this term as previously postulated.[1]

Table I

Output and efficiency for runs of varying recirculation

Control temperature, ° F	Output, lb. C.D.S./h./sq. ft.	Efficiency %
160	1·6	32·2
180	1·9	31·1
200	2·1	29·5
220	2·3	27·0

Discussion

The technique of handling the kelp for these tests lacked the simplicity required in a commercial operation and it was not possible to improve efficiency by adding one or more banks of kelp. These limitations were due to the small size of the dryer. The results of these tests show, however, a suitable method of drying and provide data for the design of a commercial through-circulation dryer for kelp.

Conclusions

Pilot-plant tests on through-circulation drying of *L. longicruris* have been conducted with a batch dryer. Results show that whole weed may be dried satisfactorily when hung parallel to the direction of air flow.

Efficiency can be improved by recirculating air toward the end of the run. It has been found that, for even drying, a reversal of air flow about half-way through the run is desirable.

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NITROGEN FIXATION IN EXTRACTS OF *AZOTOBACTER VINELANDII* *

By D. J. D. NICHOLAS† and D. J. FISHER

When cells of *Azotobacter vinelandii* (O) strain grown for 18 h. at 30° were disrupted in the culture medium in which they were grown, either by lysozyme treatment or by the ultrasonic probe, they incorporated nitrogen-15 in amounts that were comparable to those assimilated by whole cells. Even cell-free extracts prepared by centrifuging the treated cells in the culture medium at 25,000 *g* for 30 min., showed a significant enrichment of the stable isotope, viz., between 0·22 and 0·54 atom-% excess, which is more than 100 times the values recorded previously. This probably constitutes the first unequivocal demonstration of nitrogen fixation in cell-free extracts of a nitrogen-fixing organism.

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Introduction

It is well established that soil micro-organisms, including species of *Azotobacter* and *Clostridia*, fix atmospheric nitrogen. Although several attempts have been made to demonstrate the fixation mechanism in extracts of the micro-organisms the results have been variable and of doubtful significance.[1–4] Even protoplasts prepared from *Azotobacter* by careful lysis of the cell walls with lysozyme incorporated only small amounts of ^{15}N, viz., an enrichment of 0·04 atom-% excess.[5] These negative results over a period of years have led investigators to the conclusion that nitrogen fixation can only occur in intact bacterial cells and that once they are disrupted the fixation process no longer functions.

In this paper methods are described for preparing cell-free extracts of *Azotobacter vinelandii* (O) strain, that will incorporate ^{15}N at a measurable rate. The cells must, however, be disrupted in the culture medium in which they were grown. Preliminary results of this work have already been reported.[6]

Experimental

Culturing the bacterium

Azotobacter vinelandii (O) strain, kindly supplied by Dr. R. Burris and Dr. P. Wilson, Wisconsin University, Madison, U.S.A., was grown in 500-ml. amounts of the following nitrogen-free medium contained in 1-l. Erlenmeyer flasks: sucrose 20 g.; KH_2PO_4 0·16 g.; K_2HPO_4 0·64 g.; $MgSO_4,7H_2O$ 0·2 g.; NaCl 0·29 g.; $CaSO_4,2H_2O$ 0·05 g.; $Na_2MoO_4,2H_2O$ 4 mg.; $FeSO_4,7H_2O$ 15 mg.; deionised water to 1 litre from Deminrolit plant Mark IV (pH of culture medium 7·2). The cultures, well-aerated with sterile air, dispersed in the medium through sintered discs, were grown in shake culture in an incubator at 30°. The bacterium, subcultured weekly, was maintained on nutrient agar slopes as described elsewhere.[7]

Preparation of bacterial extracts

After 24 h. growth when the optical density measurements of 3-ml. aliquots of the growing culture in a 1-cm. spectrophotometer cell varied between 0·5 and 0·8 at 660 mμ, the bacteria were disrupted. This was done either by adding egg-white lysozyme or with the ultrasonic probe.

Lysozyme treatment.—This was a modification of Repaske's method[8] for lysing Gram-negative bacteria but instead of the 2-amino-2-hydroxymethylpropane-1,3-diol (tris) buffer, sodium pyrophosphate was used since the former contains sufficient nitrogen to interfere seriously with the subsequent determination of ^{15}N. The method is as follows: 50 ml. of culture medium containing the bacteria are placed in a 100-ml. Erlenmeyer flask and the pH adjusted to 8·0 with 0·01N-NaOH and the following reagents added: 0·1M-sodium pyrophosphate (pH 8·0) 5 ml., disodium ethylenediaminetetra-acetic acid (EDTA) (0·16 mg./ml.) 5 ml. and egg-white lysozyme (0·2 mg./ml.) 5 ml. By taking aliquots of this solution every 5 min. the progress of the lysis was followed in a spectrophotometer at 660 mμ in 1-cm. cells. Disruption of the cells was almost complete after 2 hr. The pH was then adjusted to 7·2 with 0·01N-HCl.

Ultrasonic treatment.—A Mullard ultrasonic probe (20 kc/s) was used as follows: 30 ml. of culture medium containing the cells were put in a double-wall glass vessel (40 ml. capacity) which was kept cold by circulating iced water through the outer jacket. The contents were agitated slowly with a magnetic stirrer and the cells subjected to ultrasonic treatment for 15 min. The probe was inserted approximately $\frac{1}{8}$ in. below the surface of the cell suspension.

Cell-free extracts.—These were prepared by centrifuging the preparations, from either of the above treatments, at 25,000 *g* for 30 min. in a refrigerated centrifuge at 4°. Microscopical examination and plating experiments confirmed the complete absence of whole cells from these extracts.

Nitrogen-15

The stable isotope of nitrogen was obtained from A.E.R.E., Harwell, as $^{15}NH_4NO_3$, 32 atom-% excess, and later, when no further supplies were available from Harwell, from Eastman Kodak Company, Rochester, U.S.A., in the same form but with a greater enrichment of the ammonium radical, viz., 64 atom-% nitrogen excess. The latter was diluted with carrier ammonium nitrate to 32 atom-% excess. The $^{15}NH_4NO_3$ was converted to $^{15}N_2$ gas by heating

it in a special apparatus in the presence of NaOH and circulating the ammonia formed several times through a column of CuO at 650°, as described elsewhere.[9]

A gas mixture was prepared by mixing $^{15}N_2$ with O_2 and He at atmospheric pressure in 1-l. flask in the proportions $^{15}N_2$ 30, O_2 30, He 40%. In later experiments O_2 was reduced to 20%.

Exposure of cells and their extracts to nitrogen-15

Whole cells or their extracts (10 ml. aliquots) were exposed to the gas mixture in 25-ml. sterile Erlenmeyer flasks fitted with B-19 sockets having Thunberg type Quickfit stoppers, as shown in Fig. 1. The flasks were first evacuated to 0·1 mm. Hg and then the gas mixture introduced at atmospheric pressure, by water displacement. The Thunberg stoppers were then sealed and the flasks put in a reciprocator for 18 h. (80 oscillations/min.) in an incubator at 30°. As similar enrichment values were obtained after 2 or 3 h. exposure to the tracer, these shorter periods were used since bacterial contamination might occur at longer exposure times.

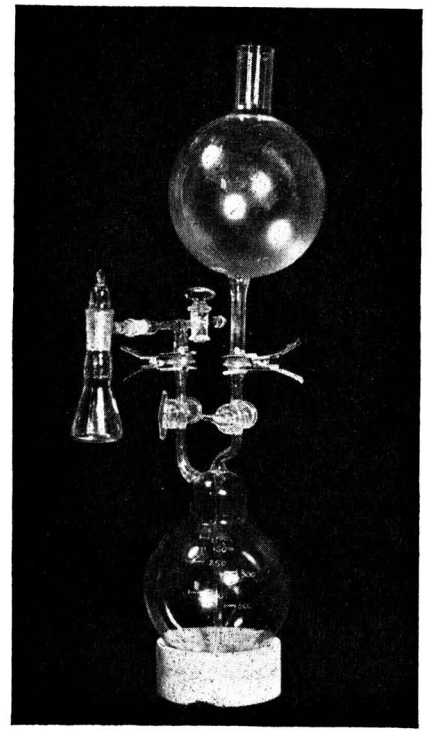

FIG. 1.—*Glass apparatus used to expose suspensions of cells or their extracts to the gas mixture* $30N_2/20\ O_2/40\ He$, *containing* ^{15}N

Determination of nitrogen uptake

The cell suspensions or cell extracts after exposure to ^{15}N were digested for 6 h. in micro-Kjeldahl flasks with 0·5 g. of catalyst mixture (HgO 4 g.; K_2SO_4 50 g.) and 3 ml. of H_2SO_4 A.R. (sp. gr. 1·84), on a thermostatically controlled electric coil heater. After digestion, the solution was diluted to 25 ml. with distilled water, and an aliquot taken for the determination of total N in a Markham apparatus. Subsequently another aliquot, containing between 30 and 400 μg. of N was distilled, in the same apparatus, into 5 ml. of 0·07N-H_2SO_4 in a boiling tube. The distillate which was reduced to 1 ml. in a water-bath, was transferred into one of the limbs of a Rittenberg tube; into the other arm was put 1 ml. of alkaline sodium hypobromite previously treated with 0·1% w/v KI to prevent the formation of oxygen.[10] The Rittenberg tube was evacuated, first to 0·1 mm. Hg with a vacuum pump and then to 1×10^{-5} mm. Hg with a mercury diffusion pump.

Measurements of the nitrogen peak heights at mass numbers 28, 29 and 30 and the one for oxygen at 32 was made in a mass spectrometer. In addition to determining the N and O peaks, argon (40) was also used as a reference peak.[11] The % enrichment of ^{15}N in the samples was calculated from the ratio $\frac{N_2^{29} + 2(N_2^{30})}{2(N_2^{28} + N_2^{29} + N_2^{30})}$ as described elsewhere.[12]

Results

Lysozyme treatment

Harvested cells.—Experiments were made to determine the optimum conditions for lysis of the bacteria. The results in Fig. 2 show that cells of *Azotobacter*, collected at 4° in a Sharples centrifuge and washed with 0·85% w/v NaCl, may be lysed by suspending them in 0·01M-sodium pyrophosphate containing sucrose, phosphate, sodium-EDTA and lysozyme. The pH for disruption of the cells appears to be optimal between pH 7·8 and 8·0, outside these values lysis fell off abruptly. Although lysis in the pyrophosphate buffer proceeded at a lower rate to that in the tris buffer used by Repaske,[8] the final lysate was similar in both.

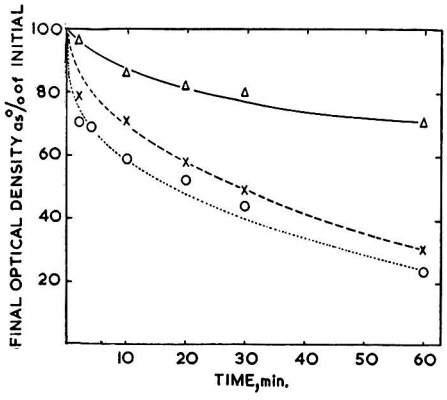

FIG. 2.—*Effect of pH on the harvested cells of* Azotobacter (18–20 h. growth)

Cells (total N ≡ 3 mg.) suspended in 40 ml. of 2% w/v sucrose in 0·01M-phosphate (buffer at the pH indicated); 5 ml. of 0·1M-sodium pyrophosphate; 5 ml. disodium-EDTA (0·16 mg./ml.); 5 ml. egg-white lysozyme (0·2 mg./ml.); 3-ml. aliquots of this mixture taken at various time intervals in a 1-cm. cell, and the optical density measured in a spectrophotometer at 660 mμ (decrease in optical density indicates lysis of the cells).

△——△ pH 7·2
× — — — × pH 7·8
○ - - - - - ○ pH 8·0

When the lysed cells were exposed to a gas mixture containing ^{15}N at pH 7·2, there was no significant incorporation of the tracer.

Cells in culture medium.—Attempts were then made to effect lysis of the cells in the culture medium in which they were grown. The results presented in Fig. 3 show that disruption of cells in the media was slower than for harvested cells. A narrow pH range, viz., 7·8 to 8·0, was again optimal for lysozyme action. The marked incorporation of ^{15}N into these treated cells is, however, in marked contrast to the lack of fixation in those lysed in the absence of the culture medium—in fact, the rate of incorporation was comparable with that for whole cells. Table I shows that even after centrifuging the lysed cells at 25,000 *g*, there was still a substantial fixation of nitrogen in the cell-free extracts.

Ultrasonic treatment

There was no incorporation of ^{15}N into extracts of harvested cells after the ultrasonic treatment, but results in Table II again demonstrate the importance of the culture medium for nitrogen fixation. Cells treated with the ultrasonic probe, in the medium in which they were grown, showed a similar enrichment of the isotope to that for whole cells. The supernatant solution obtained after centrifuging at 25,000 *g* also incorporated the stable isotope at a measurable rate.

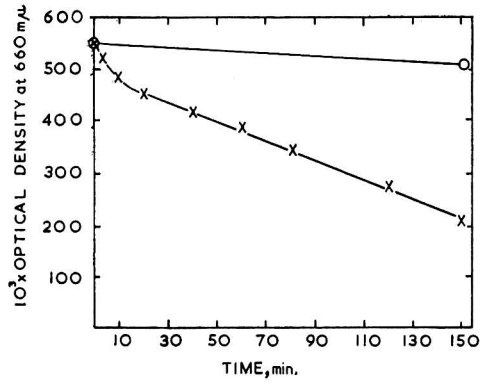

FIG. 3.—*Effect of lysozyme on the lysis of* Azotobacter *cells in the culture medium in which they were grown*
(18–20 h. growth)

Optical density of 3-ml. aliquots of the following reaction mixtures were measured in 1-cm. cuvettes in a spectrophotometer at 660 mμ: 50 ml. of culture solution plus cells, pH adjusted with 0·01N-NaOH to 8·0, then the following solutions added: 5 ml. of 0·1M-sodium pyrophosphate (pH 8·0), 5 ml. of disodium-EDTA (0·16 mg./ml.) and 5 ml. of lysozyme (0·2 mg./ml.) (decrease in optical density indicates lysis of the cells).

O——O no lysozyme
×——× lysozyme added

Table I

*Fixation of nitrogen-*15 *into extracts of* Azotobacter vinelandii *prepared by lysozyme treatment of the cells*
(atom-% ^{15}N excess)

	(1) Whole cells	(2) Lysis of cells with lysozyme. (supernatant solution used after centrifuging at 25,000 *g* for 30 min.)	(3) Boiled supernatant solution from (2)	(4) Supernatant solution from (2) treated with 10% w/v trichloroacetic acid
Expt. (i)	4·00	0·22	0	0
Expt. (ii)	3·54	0·54	0	0

Table II

*Fixation of nitrogen-*15 *into extracts of* Azotobacter vinelandii *prepared by ultrasonic treatment of the cells*
(atom-% ^{15}N excess)

(1) Whole cells	(2) Ultrasonic treatment of cells (15 min.)	(3) Supernatant solution from (2) after centrifuging at 25,000 *g*. for 30 min.	(4) Boiled supernatant solution from (2)
2·73	2·54	0·385	0

Discussion

The several attempts made to demonstrate a fixation of nitrogen gas into extracts of *Azotobacter* were invariably based on processing harvested cells collected from the culture medium. Despite the use of a variety of methods to disrupt these cells and the addition of a number of cofactors, the incorporation of ^{15}N into the cell extracts was usually insignificant. These results together with those from parallel studies with legumes where root nodules isolated from the host plants rapidly lost their ability to fix nitrogen, led investigators to the conclusion that nitrogen fixation could occur only in the intact organisms. In experiments reported here it is clear that cell-free extracts of *Azotobacter* may be prepared which incorporate measurable amounts of ^{15}N, provided the cells are disrupted in the media in which they were grown. Several points, however, remain to be investigated including the nature of contributory factors in the medium, the extent of fixation including identification of the intermediate products and the characterisation of the enzymes involved. Work is in progress to elucidate these problems, now that nitrogen fixation has been achieved in a cell-free system.

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Note added in proof

Since this manuscript was submitted, cell-free extracts prepared from other micro-organisms have been shown to fix atmospheric nitrogen. Thus Carnahan, Mortensen, Mower & Castle (*Biochim. biophys. Acta*, 1960, **38**, 188) obtained a substantial fixation of nitrogen in extracts of *Clostridium pasteurianum* especially when pyruvate was added. Schneider, Bradbeer, Singh, Wang, Wilson & Burris (*Proc. nat. Acad. Sci., Wash.*, 1960, **46**, 726) have confirmed these results and extended them to include extracts prepared from a variety of blue-green algae and also from *Rhodosporillium rubrum*.

We have confirmed the results reported in this paper using radioactive ^{13}N and find that nitrogen is incorporated rapidly into particle-free extracts of *Azotobacter* after a 15 min. exposure to the tracer. A detailed account of this and related work is in the press.

THE TOXICITY TO RABBITS AND SOME OTHER ANIMALS OF THE FLUOROFATTY ACID PRESENT IN THE SEEDS OF *DICHAPETALUM TOXICARIUM*

By R. A. PETERS* and R. J. HALL

The long-chain fluorofatty acid in the seeds of *Dichapetalum toxicarium*, ' ratsbane ', is toxic to rabbits in a dose of 1 mg./kg. Death is delayed and usually occurs quite suddenly, probably due to a heart attack. Wild as well as tame rabbits, are poisoned by a bait of the ground seeds mixed with oats, and are more easily killed than sheep ; in this respect the compound is a better rabbit poison than sodium fluoroacetate. Some toxicity figures have been obtained for other animals. Deaths in sheep may be delayed up to 5 days, a fact which could explain some casualties of unknown cause in Africa.

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Introduction

The main fluorine-containing substance in the seeds of *Dichapetalum toxicarium* (known as ' ratsbane ') has been characterised recently as a fluoro-oleic acid (Peters & Hall ;[1] Peters, Hall, Ward & Sheppard[2]). Some tests of toxicity on rats during the purification and several on the purified substance indicated that 7–9 mg./kg. killed all the animals. Other tests of toxicity to rabbits, sheep, and some other animals are presented here. Owing to the difficulties involved in preparing much of the pure material, most toxicity tests upon animals have been done with the fraction of the seed fat which is soluble in acetone at −20°. The main fluoro-fatty acid in this fraction is ω-fluoro-oleic acid, the concentration of which was determined by fluorine analysis. Thus the two samples of the partially purified but still crude oil used in these tests contained 18·8% and 23·8% respectively of fluoro-oleic acid and actual doses per kg. of body weight have been calculated on the basis of these values.

In these experiments no attempt has been made to estimate the toxicity exactly, as this would have required much more of the material and many more animals. At present, it seemed important only to determine the order of toxicity. As these fluoro-acids induce accumulation of citric acid in certain tissues, this was estimated in several instances according to the method of Taylor.[3]

Experimental and results

It seems clear from Table Ia that the dose killing all our rats was slightly above 7 mg./kg. It is characteristic that the heart was rapidly attacked by the higher doses ; it became much slowed and often irregular. The breathing was also laboured and the amounts of citric acid were increased in the kidney and lung as well as in the heart. Results of tests upon guinea pigs and pigeons are shown in Tables Ib and Ic, and on rabbits and sheep in Tables II and III.

Table Ia

Toxicity of fluoro-oleic acid to rats

(doses were injected intraperitoneally in approximately 0·2 ml. of arachis oil ; the oil caused no disturbance where given alone)

Dose, mg./kg.*	No. of animals	Wt., g.	Deaths	Other effects
4	4 ♀ 1 ♂	190–220	0/5	Two showed early convulsions and one an irregular heart beat
7–12	5 ♂	150–280	4/5	The one survivor had a convulsion, but recovered
	5 ♂	80–90	2/2	
13	5 ♂	230	5/5	
23	3 ♂	200	3/3	
	2 ♂	140–170	2/2	

* Calc. as fluoro-oleic acid

Table Ib

Toxicity of fluoro-oleic acid to guinea pigs

(wt. 500–600 g. ; all male ; 2 animals in each test)

Dose, mg./kg.	Deaths
0·5	0/2
1·0	0/2
2·0	2/2
4·0	2/2

Table Ic

Toxicity of fluoro-oleic acid to pigeons

(wt. 370–590 g.)

Dose, mg./kg.	No. of animals	Deaths	Comments	Citric acid, μg./g.
2	4	0/4		
3	7	5/7	Death at 6 h. 30 min.	Heart 620, kidney 305, lung 218
			Deaths at 6 h. 55 min. and 2 h. 15 min.	
			Death at 2 h. 20 min.	Heart 1050, kidney 183, lung 309
			Death at 2 h. 20 min.	Heart 1175, kidney 1100, lung 307
5	1	1/1	Death at 2 h. 8 min.	
10	1	1/1	Death at 1 h. 30 min.	Heart 1840, lung 308

Table II

Toxicity of fluoro-oleic acid to sheep

breed : Clun Forest, all male

(doses were given in gelatin capsules placed well to the back of the throat)

	Dose, mg./kg.	Weight, kg.	Comments
1.	5·0	33·7	Died in 3 days. Heart rate : 1st day 129/min. ; 2nd day 96/min. Citric acid μg./g. heart 1012, kidney 1120, lung 373
2.	3·0	40·3	Quite well for 4 days ; found dead on 5th day.
3.	2·0	30·87	Went off food on second day, then appetite improved. At 1.0 p.m. on 5th day weighed and seemed well, but found dead 3 h. later
4.	2·0	68·2	Died suddenly between 6 and 8 a.m., 20 h. after dose. At post mortem, heart showed haemorrhagic patches
5.	2·0	84·8	Well after 7 days
6.	1·0	40·78	These three sheep lived without mishap for 16 months
7.	1·0	40·32	
8.	1·0	43·8	
9.	1·0	57·8	Well after 7 days
10.	1·0	83·7	Well after 7 days

Table III

Toxicity of fluoro-oleic acid to rabbits

(dose given by intraperitoneal injection except where indicated)

Dose, mg./kg.	No.	Wt., kg.	Deaths	Effect	Citric acid, μg./g. Heart	Kidney	Liver	Brain	Lung
Tame									
0·25	1 ♀	2·80	0/1						
0·5	4 ♀ 1 ♂	2·70–4·40	3/5	Deaths at 3 h. 30 min., over 7 and under 24 h.	730	209	57	48	109
	4 ♀	2·70–3·15	2/4	Deaths within 20 h. and 12 h.					
1·0	4 ♀	3·93 : 3·33 (injected)	4/4	Injection : deaths in 2 h.					
		4·70 : 4·90 (oral)		Orally : deaths in 6 h. 45 min. 4 h. 40 min.	310	99	29	47	98
	2 ♀	3·20 : 3·60	2/2	Deaths at 20 h., 4 h. 30 min.	533	792			273
2·5	1 ♂ 1 ♀	2·75	2/2	Deaths at 1 h. 10 min.,	788	—	505	72	420
		3·15		4 h. 40 min.	1210	770			543
Wild									
1·2	2	1·56	2/2	Deaths at 4 and under 18 h.					
		1·61							
1·18	1	1·36	1/1	Death under 18 h.					
0·5	2	1·41	1/2	Death within 24 h. Surviving animal killed and tissue citrate levels determined	1370	167			221
		1·50							

(No rabbit has survived a dose of 1 mg./kg.)

Table IV, giving results of tests by feeding for tame and wild rabbits, shows that the poison was equally active in oral tests using the oil mixed with oats or other cereals ; the wild rabbits, tested in the open, ate the bait and were killed by the ground seeds mixed with oats. It is particularly striking that death is sudden, suggestive of a heart attack. We have seen an animal die while actually feeding on cabbage after ingestion of poisoned oats. As might be expected for a long-chain fluorofatty acid, the accumulation of citric acid in the heart is relatively high as compared with the kidney (Peters[4]). Normal values do not exceed 90 μg./g. of fresh tissue and are usually not more than 50 μg./g.

Table IV

Toxicity of fluoro-oleic acid to tame and wild rabbits

(dose* administered in feeding stuffs)

Expt.	No. of animals	Dose and administration	Result
Tame.	Wt.: 2·7–4·9 kg.		
1	2 ♀	Crude oil in 20 g. of rabbit cake ≡ 1 mg./kg. fluoro-oleic acid per animal.	Both died in 8 h. although one ate only half feed
2	2 ♀	Expt. 1 repeated in oats instead of rabbit cake	Both dead in 6 h.
3	1 ♂ 1 ♀	1 g. of crushed seeds mixed with 20 g. of damp oats per animal	One died in 2 h.; the other after 24 h. but ate much less
Wild.	Wt.: 255–1610 g.		
4	2 ♂	First fed with green feed for 2 days then oats 2 days. Finally a mixture of 1 g. of seeds with 20 g. of oats per animal	Both dead next day. As 39 g. of feed were left, each animal had eaten less than 0·5 g. of seeds
5	1 ♀	Oats and cabbage 14 days; then 1 g. of seeds mixed with 20 g. of oats	Died within 1 day
6	2 ♂ ♀	Oats and cabbage 3 days; then 500 mg. of seeds in 30 g. of oats per animal	Both dead within 1 day. 35 g. of feed left
7	1 ♀	Oats and cabbage 3 days; then 0·5 g. of seeds in 30 g. of oats	Died in 2 days
8	3 ♀♀♀ 1 ♂	Oats and cabbage 5 days; then 250 mg. of seeds with 5 g. of oats per animal	3 dead within 1 day, the 4th in 2 days
9	1 ♂ 2 ♀♀	Oats and cabbage 1 day; then 170 mg. of seeds in 7 g. of oats per animal, then a further similar dose in 24 h.	One died after first dose; the two others after 2 doses
10	3 small ♀ (255–281 g.)	Oats, cabbage and hay 10 days, then 35 mg. of seeds in 7 g. of oats per animal	Dead within 24 h., oats found in stomachs
	1 ♂ 2 ♀♀ (large) (1 pregnant)	40 mg. of seeds in 5 g. of oats per animal	Pregnant rabbit dead within 24 h. Citric acid: heart 2110 μg./g., kidney 1020 μg./g. One female dead within 24 h. Male dead within 48 h.

* 1·0 g. of ground seeds contained approximately 32 mg. of fluoro-oleic acid calculated from the fluorine analysis

Discussion

In terms of fluorine, this fluorofatty acid is approximately three times more toxic to the rabbit than is fluoroacetic acid and two to three times less toxic to the sheep as judged by the figures quoted by Meldrum *et al.*,[5] i.e., the dose of sodium fluoroacetate killing all animals being: rabbit, 0·7–0·8 mg./kg.; sheep, 0·45 mg./kg. From the LD_{50} results in Table V, it will be seen that the ratio of the dose of fluoro-oleic acid for the rabbit as compared with that for the sheep is much more favourable than for fluoroacetate so that there should be less risk of killing sheep.

Table V

Estimates of LD_{50} (mg./kg.) for fluoro-oleic acid

Animal			LD_{50}
Rat	>4	<7	6
Guinea pig	>1	<2	1·5
Pigeon	>2	<3	2·5
Rabbit	>0·25	<1	0·5
Sheep	>1·0	<3	2·0

Dose ratio rabbit/sheep 1/4

Estimates by Meldrum *et al.*[5] for sodium fluoroacetate give for the rabbit a dose killing all tested of 0·7/0·8 mg./kg. and an LD_{50} of 0·3 mg./kg. For sheep, the dose killing all tested was 0·45 mg./kg., from which the estimated LD_{50} may be about 0·25 mg./kg. This makes the dose ratio of fluoroacetate for rabbit/sheep approximately 1/1.

Since the ground seeds can be given, the compound should be readily available. It can be administered either as the ground seeds or as the crude oil obtained by extraction; in both cases it is best to mix with oats as recommended in Australia for sodium fluoroacetate.

Since the problem of eliminating rabbits without causing suffering is urgent in Australia and New Zealand[6, 7] and may return very soon in the U.K.[8] now that resistance to myxomatosis appears to have set in, it is suggested that it is worth examining whether oats treated with the ground seeds of *D. toxicarium* or with crude fluoro-oleic acid may not be a better way of killing rabbits, and be relatively safer for sheep. The death appears to be a humane one, apparently due to a sudden heart attack; and it is an additional advantage that the effect of the poison may be delayed for some hours.

At the same time, it should be recognised that the toxic compound must be kept away from cats and dogs, as in a few tests we have confirmed that it is more toxic to these than to rabbits.

In the experiments on sheep, one point of scientific as well as practical interest emerged. In at least two cases (sheep 2 and 3), the animal appeared to be quite well to within 3 h. of its death; which was so unexpected that no observations were made during this period. This raises the question how the fluoro-compound was retained for 4 days without access to tissues which could convert it to fluorocitrate and whether such retention could be intestinal. Nevertheless, the fact that this retention can happen gives the first logical grounds for explaining sudden deaths among cattle reported from East Africa, where no pathological cause was discovered. It would be possible for an animal to eat some seeds and wander for 2 to 3 days in the bush before its sudden death.

Acknowledgments

The authors' thanks are due to Miss R. Shawdon (now Mrs. Jourdan) for technical help and to Mr. J. Clark for assistance with the sheep. They are indebted to Mr. R. F. Furze who farms at Ashdon, Essex, and Mr. H. Naylor, a pest operator of the Ministry of Agriculture, Fisheries and Food, for supplying wild rabbits. They are grateful to the Director and staff of the Tropical Products Institute, London, for the supply of the ground seeds of *D. toxicarium* and to those in Sierra Leone who collected them; and also to Dr. J. H. Gaddum, F.R.S., for his helpful suggestions in the presentation of results.

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ERRATUM

In the paper by Hemingway (*J. Sci. Fd Agric.*, 1960, **11,** 355) 'Mean % Na' in line 10 on p. 360 should read 'Mean % Ca'.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

OCTOBER, 1960

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

OCTOBER, 1960

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Soils of Georgia. J. Giddens, H. F. Perkins and R. L. Carter (*Soil Sci.*, 1960, **89**, 229—238).—The formation and distribution of soil types of the area are described and discussed in conjunction with the topographical and climatological factors. T. G. MORRIS.

Lateritic [A, B] soils of the southeastern portion of the Australian arid zone. R. W. Jessup (*J. Soil Sci.*, 1960, **11**, 92—105, 106—113).—Characteristics of the soils are presented. A. H. CORNFIELD.

Soils of the Queen Elizabeth Islands, Canadian Arctic. N. J. McMillan (*J. Soil Sci.*, 1960, **11**, 131—139).—Two well-drained profiles and one water-logged profile are described. Profile development due to chemical weathering and plant action was negligible in this arctic environment. These soils should properly be regarded as polar equivalents of lithosols and regosols and should not be classed with the Tundra Great Soil Group. A. H. CORNFIELD.

Saline soils in the Kirkuk Plain, Northern Iraq. S. A. Harris (*J. Soil Sci.*, 1960, **11**, 114—130).—Characteristics of the soils are presented. A. H. CORNFIELD.

Podsols in the vicinity of the Nelchina and Tazlina glaciers, Alaska. J. Kubota and L. D. Whittig (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 133—136).—Characteristics of a no. of profiles are described. The soils appear to be related to the northern dwarf podsols or nanopodsols. A. H. CORNFIELD.

Composition of colloids in the soils of Israel. S. Ravikovitch, F. Pines and M. Ben-Yair (*J. Soil Sci.*, 1960, **11**, 82—91).—Chemical and mineralogical composition data are presented. A. H. CORNFIELD.

Red earths and colloids of the red earths. L. E. Lisanti (*J. Soil Sci.*, 1960, **11**, 77—81).—Chemical characteristics of red earth soils and colloids separated from them are presented for samples from various parts of Italy. A. H. CORNFIELD.

Worm-worked soils of Eastern South Dakota, their morphology and classification. G. J. Buntley and R. I. Papendick (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 128—132).—Characteristics and distribution of soils which show nearly complete destruction of zonal profile horizontation as the result of mixing by intensive worm activity are described. The name "Vermisol" is suggested for such soils. A. H. CORNFIELD.

Post-glacial sediments as a record of regional soil drifts. W. H. Pearsall, J. Gay and J. Newbould (*J. Soil Sci.*, 1960, **11**, 68—76).—Analytical data are presented for N, loss on ignition, and mineral elements for deep-water sediments from Esthwaite Water in the English Lake District representing different depths below the mud surface and sediments of ages back to and including the late-glacial, of which the pollen status is known. Results are discussed in relation to the regional changes in soils from the lake drainage systems during the post-glacial period. A. H. CORNFIELD.

Butana grass patterns. B. P. Ruxton and L. Berry (*J. Soil Sci.*, 1960, **11**, 61—62).—An additional possible mode of origin of the Butana grass patterns (cf. *ibid.*, 1959, **10**, 34) is presented. A. H. CORNFIELD.

Tree patterns in the Sudan. G. A. Worrall (*J. Soil Sci.*, 1960, **11**, 63—67).—Bands of acacia trees occur in the Sudan in patterns resembling the Butana grass patterns (*ibid.*, 1959, **10**, 34). They occur only in the red sand belt of Western Sudan and they lie on gentle slopes very roughly on the contour. Possible reasons for the occurrence of the patterns are discussed. A. H. CORNFIELD.

Infiltration rates in frozen soils in Northern Minnesota. J. H. Stoeckeler and S. Weitzman (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 137—139).—Concrete-type frosted soils (impermeable to air), which had very low infiltration rates, were most prevalent in sod lands. In forest soils the porous-concrete frost type was usually present, and these and partly frozen soils showed much higher infiltration rates. Unfrozen soils showed about five times the infiltration rate of porous-concrete frosted soils. A. H. CORNFIELD.

Kinetics of moisture flow into unsaturated soils. J. W. Biggar and S. A. Taylor (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 81—85).—A marked temp. coeff. for the rate of water uptake by horizontal columns of dry soil was interpreted in terms of activation energies, which were of the order of 1—3 kg.-cal. per mole of water entering the soil. A. H. CORNFIELD.

Measurement of soil anisotropy with piezometers. T. Talsma (*J. Soil Sci.*, 1960, **11**, 159—171).—Anisotropy values obtained by measurements with piezometers of different sizes are presented. Values varied with cavity dimensions below the piezometers, the large piezometers giving anisotropy ratios <1, the small piezometers generally >1. A. H. CORNFIELD.

Soil structure and fabric; their definition and description. R. Brewer and J. R. Sleeman (*J. Soil Sci.*, 1960, **11**, 172—185).—The paper defines concepts and terms to cope with the description of the physical constitution of soil materials having regard to current concepts used in pedology and geology. A. H. CORNFIELD.

Effect of pH on the wet strength of soil crumbs. W. W. Emerson and M. G. Dettmann (*J. Soil Sci.*, 1960, **11**, 149—158).—The wet strength of clay crumbs due to attractive forces between clay particles was greater in acid than in corresponding calcareous soils. The cementing effect of trivalent cations was dominant in very acid soils in which Al^{3+} could exist in the soil solution. There was no evidence of cementing due to pptd. $Al(OH)_3$ or $Fe(OH)_3$. The same concn. of $CaCl_2$ was required to flocculate suspensions of moderately acid surface soils at the natural pH and after buffering the suspensions to pH 7·4. Clay and soil flocculated at lower $CaCl_2$ concn. when free from org. matter. A. H. CORNFIELD.

Turbidimeter technique for measuring the stability of soil aggregates in a water–glycerol mixture. J. M. Davidson and D. D. Evans (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 75—79).—Air-dried soil (10—30 g.) is uniformly mixed with water–glycerol (1 : 9) in a 1-l. test tube and the density of the suspension measured in a photoelectric turbidimeter over a period of time. The mean wt. diameter of the soil aggregates is determined by plotting the log. of the potentiometer readings against the square of the aggregate diameter and measuring the area under the curve. The method measures differences in structure not measured by the usual wet-sieving method. A. H. CORNFIELD.

Effect of synthetic soil-aggregating chemicals on some physical and biological properties of certain Ohio soils. J. D. DeMent (*Dissert. Abstr.*, 1960, **20**, 3000—3003).—The following substances were applied to soils: Ca salt of vinyl acetate maleic acid copolymer, hydrolysed polyacrylonitrile, isobutyl maleic acid copolymer, carboxymethylcellulose. All caused an immediate increase in % of soil aggregates $>0{\cdot}25$ mm. in diameter, and in all except fine-textured soils increased microbial activity, as measured by output of CO_2, ammonification and nitrification. Soil texture influenced microbial activity more than did soil-aggregating substances. Yields and N contents increased in the second, but not in the first, cutting of red clover grown on treated soils. Surface applications of soil-aggregating substances in liquid form improved the emergence of seedlings in about 50% of cases, especially with small seeds, and when heavy rain fell between sowing and emergence. M. D. ANDERSON.

Moisture control techniques for experimental field plots. L. Lyles and N. P. Woodruff (*Agron. J.*, 1960, **52**, 298—299).—Methods for the control of soil moisture in field plots by protecting them from rainfall with polyethylene and Al sheeting are described. A. H. CORNFIELD.

Iron and the E_h of waterlogged soils with particular reference to paddy. J. W. O. Jeffery (*J. Soil Sci.*, 1960, **11**, 140—148).—When adjusting soil E_h measurements for pH variations the term $pH \times 3 \times 2{\cdot}303RT/F$, which is 3 times greater than that normally used, is recommended on the basis of tests with waterlogged mixtures of soil and org. matter. A. H. CORNFIELD.

Micro-determination of cation-exchange capacity and total exchangeable bases. L. C. Blakemore and A. J. Metson (*Soil Sci.*, 1960, **89**, 202—208).—The method is based on the ammonium acetate method commonly in use on the macro-scale. Soil (20 mg.) is leached with four portions of 0·5 ml. of N-NH_4 acetate, the leachings being used to determine total exchangeable bases. The leached soil is washed free of the leaching solution with alcohol and then transferred quant. to a Conway microdiffusion chamber in which NH_4^+ content is determined. The cation-exchange capacity so determined agreed reasonably well with that obtained by the macro-technique. T. G. MORRIS.

Characterisation of exchange reactions of strontium or calcium on four clays. W. R. Heald (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 103—106).—The adsorption isotherm was used to demonstrate two independent sites for adsorption of Sr or Ca on bentonite, illite, vermiculite and kaolin. The exchange of Sr and Ca was stoicheiometric on both sites. Sr was adsorbed more tightly than Ca on either site, but the difference decreased with decreasing exchange capacity of the clays. A. H. CORNFIELD.

Chemistry of Comber's test. S. C. Chang and M. P. Feng (*Soil Sci.*, 1960, **89**, 219—222).—Ferric thiocyanate is stable below pH 3 and the colour is independent of pH but directly proportional to the concn. of Fe^{3+}. At pH >5 the colour disappears. Between pH 3 and pH 5 the colour is directly proportional to the concn. of Fe^{3+} and inversely proportional to the pH. Hence Comber's test measures soil acidity only at pH 3–5. Soils with pH >5 give a colour which is due to the replacement of exchangeable H in the clay by K from the KCNS resulting in a lower acidity for soil–KCNS solution than for soil–water. If the exchange capacity of the soil is high the fall in acidity will also be high. Comber's test depends upon the pH of the soil, the exchange capacity and the amount of solid Fe compound in the soil. Lime requirement is related to the first two properties only. T. G. MORRIS.

Reaction efficiencies of liming materials as indicated by lysimeter leachate composition. W. M. Shaw and B. Robinson (*Soil Sci.*, 1960, **89**, 209—218).—In lysimeter experiments the efficiencies of a high-grade calcitic marble, a high-grade dolomite and a phosphate furnace slag for neutralising an acid loam were compared. With practically every treatment the outgo of Ca and Mg was greatest in the first year, thereafter falling to a fairly constant figure. A correlation of computed supplies of Ca + Mg (based on carbonate dissolution in the soil) with the (Ca + Mg) outgo in the first year gave a constant ratio for this soil. Using this constant, the results from one year's lysimeter leachate outgo could be used to assess the neutralising efficiency of any limestone. Outgo of K, N and S was highest in the first year, thereafter reaching a constant level. The finest particle size of liming material caused the lowest K outgo and, in general, the highest N outgo, S being unaffected. The ratio of (NO_3^- + SO_4^{2-}) to the total cation outgo was an index of sufficiency of liming. A high Al outgo followed a high NO_3^- + SO_4^{2-}/total cation output. T. G. MORRIS.

Techniques and accessories for adaptation of the gas chromatograph to soil nitrogen studies. D. H. Smith and F. E. Clark (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 111—115).—Equipment and techniques are described for separating and measuring N_2, N_2O, NO, NO_2, NH_3, O_2 and CO_2 in soil atm. A. H. CORNFIELD.

Factors affecting volatilisation of ammonia formed from urea in the soil. J. W. Ernst and H. F. Massey (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 87—90).—Loss of NH_3 from a silt loam treated with urea increased with temp. and pH. The loss from soil of pH 6·5 was very similar when urea was topdressed, mixed with the top 0·25 in. of soil, or topdressed and watered into the soil with 0·25 in. water. Loss of NH_3 increased with increasing initial soil moisture content. Although NH_3 was lost without concurrent drying of the soil, the greatest losses occurred when moisture also was being lost. A. H. CORNFIELD.

Native fixed ammonium and fixation of applied ammonium in several Wisconsin soils. L. M. Walsh and J. T. Murdock (*Soil Sci.*, 1960, **89**, 183—193).—A mixture of N-HF and N-HCl effectively removed all fixed NH_4^+ from soil when shaken for 24 hr. with the soil in the proportion 40 : 1. All soils studied contained some fixed NH_4^+, coarse textured soils having less than silt loams. Under moist conditions most soils fixed small amounts of NH_4^+; the B horizon of grey-brown podsolic soils fixing about twice as much as the surface layers. Brunizem soils varied considerably in their ability to fix NH_4^+ and generally were less able to do so than podsols. On the average, freezing the soil after addition of NH_4^+ resulted in twice as much fixation as that under moist conditions. Oven-dried soils fixed more than frozen soils. Relationships between exchangeable K, total N and fixed NH_4^+ were studied. T. G. MORRIS.

Mechanism of surface nitrate accumulation in a bare fallow soil in Uganda. J. R. Simpson (*J. Soil Sci.*, 1960, **11**, 45—60).—Nitrate accumulation which occurred in a fallow red medium loam latosol during dry periods and which amounted to 70 p.p.m. of NO_3^--N is mainly due to bacterial nitrification, which occurred very rapidly under gradual drying conditions and continued until the moisture content fell to <8%. The "classical" nitrifying organisms were present in this soil. Upward movement of NO_3^- by diffusion or capillary transport may play a limited part in NO_3^- accumulation in the topsoil. After heavy rainfall NO_3^- is leached downwards and is also probably lost by bacterial denitrification under the temporary reducing conditions which prevail. There was no evidence that photochemical nitrification occurred in this soil. Shading or mulching the soil reduced NO_3^- accumulation. A. H. CORNFIELD.

Explanation of nitrification patterns observed when soils are perfused with ammonium sulphate. L. G. Morrill (*Dissert. Abstr.*, 1960, **20**, 3005).—Perfusion of 116 soils with $(NH_4)_2SO_4$ showed four nitrification patterns: (i) in soils of pH >7·3, NO_2^- accumulates rapidly, and NO_3^- does not appear until most of the NH_3 is oxidised to NO_2^-; (ii) with pH 5·5—7·3, NO_2^- is produced slowly and is rapidly converted into NO_3^-; (iii) in soils with pH <5·7 accumulation of NO_3^- is slow; (iv) some soils do not produce NO_2^- or NO_3^-. N may be lost from acid soils, but owing to low concn. of NO_2^- the loss is not large. M. D. ANDERSON.

Chemical composition of the soil in a factorial experiment with citrus. I. Exchangeable metal cations and their effect on the cation content of citrus leaves. H. Groenewegen and D. Bouma. **II. Extractable ammonium.** H. Groenewegen and J. Connor (*Aust. J. agric. Res.*, 1960, **11**, 208—222, 223—235).—I. In a semi-arid area, spray-irrigated plots under tillage, sod, and bare-surface cultivation were treated with $(NH_4)_2SO_4$ in various quantities. The effect on the exchangeable Ca, K and Mg of the soil is recorded and discussed. There was no effect on the cation content of the citrus leaves. (28 references.)

II. The changes and variation in NH_4^+ and NO_3^- contents at different soil levels are recorded. The reasons for the differences are discussed. (16 references.) S. G. AYERST.

Stability of dicalcium phosphate dihydrate in aqueous solutions and solubility of octacalcium phosphate. E. C. Moreno, W. E. Brown and G. Osborn (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 99—102).—The hydrolysis of $CaHPO_4,2H_2O$ (**I**) in dil. H_3PO_4 and in water was investigated at 25° in the absence of CO_2. $Ca_4H(PO_4)_3.3H_2O$ (**II**) precipitated when the pH of the solution exceeded 6·38. The extent of hydrolysis increased with initial pH of the solution. The solution composition followed the metastable solubility isotherm for **I** until a singular point between **II** and **I** was reached. At this point the solution had pH 6·38 and Ca and P concn. $1·36 \times 10^{-3}$M and $2·21 \times 10^{-3}$M, respectively. The solubility product of **II** was $1·25 \times 10^{-47}$. A. H. CORNFIELD.

Solubility of dicalcium phosphate dihydrate in aqueous systems. E. C. Moreno, W. E. Brown and G. Osborn (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 94—98).—The solubility isotherm for $CaHPO_4,2H_2O$ was established in the pH range 3·5 to 7·5 by leaching columns of the material with H_3PO_4 of different concn. The solubility product of the material was $2·77 \times 10^{-7}$. A. H. CORNFIELD.

Effect of sodium bicarbonate on the solubility of phosphorus in calcareous soils. S. R. Olsen, F. S. Watanabe and C. V. Cole (*Soil Sci.*, 1960, **89**, 288—291).—Suspensions of $CaHPO_4,2H_2O$ (**I**), hydroxyapatite (HA) or soil with or without calcite were equilibrated with air of constant partial pressure of CO_2. The P solubility was a function of the pH, Ca^{2+} and the form of Ca phosphate present. In solutions containing either **I** or HA with calcite and $NaHCO_3$, the value for pH − 0·5pCa^{2+} remained constant but at levels related to the p_{CO_2}. In calcareous soils the behaviour of the $CaCO_3$ was different from that of calcite. Additions of $NaHCO_3$ caused only small changes in Ca^{2+} with concomitant small changes in P solubility. T. G. MORRIS.

Investigations of phosphate reaction products in acid soils by application of solubility criteria. B. C. Wright (*Dissert. Abstr.*, 1960, **20**, 2991—2992).—In soils, to which no P fertiliser had been applied for 5 years, the concn. of P in the soil solution could not be attributed to P adsorbed on the surface of clay particles, but was governed by the solubility of some crystalline phosphate mineral of the variscite–barandite–strengite isomorphous series. The native P of most soils was present as Fe phosphate; applied fertiliser P was also converted into Fe phosphate in nearly all soils; in two it was converted almost entirely into Al phosphate. M. D. ANDERSON.

Effect of nitrogen, sulphur and lime on utilisation of rock phosphate by lucerne. J. D. Nichols, G. M. Phibbs and L. W. Reed (*Agron. J.*, 1960, **52**, 254—257).—Application of NH_4NO_3, $CaSO_4$ and S to two silt loam soils (pH 5·5) in pot tests had no effect on the uptake by lucerne of the P of applied rock phosphate. On unlimed soils lucerne yields were increased to the same extent by rock phosphate and superphosphate, but on limed soils superphosphate was the more effective. A. H. CORNFIELD.

Isolation of organic acids and their metabolism in relation to phosphorus solubility in Miami and Wooster silt loam soils. S. M. Schwartz (*Dissert. Abstr.*, 1960, **20**, 3011—3013).—Org. acids separated chromatographically from air-dry soils included acetic and formic acids, small amounts of lactic and succinic acids, traces of isocitric acid and, probably, tartaric and a mixture of high-mol.-wt.

fatty acids. On aerobic incubation of the soils, acetic and formic acids predominated at first, but isocitric and tartaric acids increased later, with accompanying increase of P sol. in 0·5M-$NaHCO_3$ at pH 8·5. Soils treated with ground lucerne gave results similar to those from untreated controls, but soils treated with wheat straw showed no correlation between content of isocitric and tartaric acids and amount of P sol. in $NaHCO_3$. Soils taken from two-year rotation plots contained little isocitric or tartaric acid, either before or after the growth of a crop of maize. M. D. ANDERSON.

Nature and mode of weathering of soil potassium reserves. P. W. Arnold (*J. Sci. Fd Agric.*, 1960, **11**, 285—292).—The principles underlying the weathering processes in feldspars, trioctahedral (**I**) and dioctahedral micas (**II**) in which the bulk of the total K occurs, in difficultly or non-exchangeable forms, are discussed. **I** are less stable to weathering than are **II**, especially in the early stages of K depletion, the difference in stability being greater than can be accounted for by Fe^{2+} contents. (47 references.) E. M. J.

Characterisation of release of potassium from several Ohio and New York soils. W. L. Garman (*Dissert. Abstr.*, 1960, **20**, 3046—3049).—The yields and K uptakes of crops on different soils were associated; K uptakes showed wider differences, and characterised the K-supplying power of soils better than did crop yields. The amounts of K released from subsoils were in the same order as those from the surface soils of the same types. Continuous leaching of soils with 0·01N-HCl was the method of removing K that gave the closest correlation with K removed by continuous cropping. Curves of K removed were characteristic for different types of soil. Possible mechanisms for release of K are discussed. M. D. ANDERSON.

Use of ^{15}N and rubidium in nitrogen- and potassium-availability studies in Ohio soils. W. S. Murphy (*Dissert. Abstr.*, 1960, **20**, 2984—2986).—The availability to crops of N and K in (i) lucerne tops, (ii) lucerne roots, (iii) inorg. salts is examined using Rb and ^{15}N as tracers. The % recovery of N and Rb by a maize crop was in the order (iii) > (i) > (ii). Recovery of Rb increased with increasing dosage. Only a small proportion of Rb was fixed by the soil, except in the case of small doses. Recovery of N from (i) and (iii) increased, and that from (ii) decreased with increasing dosages. M. D. ANDERSON.

Influence of long-term fertility management on chemical and physical properties of a Fargo clay. R. A. Young, J. C. Zubriski and E. B. Norum (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 124—128).—Over 40 years with a four-course rotation soil org. C, N and P declined by 27% on check plots and by 20% on plots receiving manure or cereal residues. Liming every 4 years had no effect. NO_3^- production during incubation correlated with total N content of the soil. Extractable P declined appreciably in check plots, less in residue and manured plots, and increased in P-treated plots. Aggregation, porosity, density, and hydraulic conductivity were little affected by the treatments. A. H. CORNFIELD.

Aluminium in soils. IV. Rôle of aluminium in soil acidity. M. R. Heddleson, E. O. McLean and N. Holowaychuk (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 91—94).—Treating soils with increasing amounts of acid resulted in increases in both exchange acidity and extractable Al. The increases were greater for initially mildly acid than for initially strongly acid soils. Acidic, strongly-weathered soils adsorbed much less Al than did neutral, slightly-weathered soils upon treatment with $AlCl_3$. Titration of Al-resin with KOH in ethanol indicated that hydrolysis was necessary for the Al to exhibit acid properties. A. H. CORNFIELD.

Boron adsorption and release by soils. J. W. Biggar and M. Fireman (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 115—120).—The Langmuir adsorption equation described the adsorption of B (BO_3^{3-}) from solution by three out of four soils. Wetting and drying of the soil following addition of water containing B increased the max. adsorption capacity and bonding energy of the soil for B. Leaching experiments showed that B was removed from three soils by desorption, whilst a fourth soil showed release characteristics similar to that obtained from a saturated solution of a B compound. A. H. CORNFIELD.

Cobalt in nitrogen fixation by a legume. H. M. Reisenauer (*Nature, Lond.*, 1960, **186**, 375—376).—Addition of 0·1μM-Co to "purified" culture solutions increased glasshouse growth of lucerne by 66% and doubled nodule wt. during a stress period of 2 weeks. The non-inoculated plants showed N-deficiency symptoms. In presence of *Rhizobia* and 0·5mM-NaCl, Co supplements increased the fixation of N by the plants. (20 references.) W. J. BAKER.

Sodium fixation in salt-affected soils. K. L. Babcock (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 85—86).—Studies with ^{22}Na added to salt-affected soils showed exchange between soil and added Na of some Na which was non-exchangeable with Ca^{2+} or NH_4^+. There was evidence that a solid Na compound exists in these soils which dissolves in cation-exchange extracting solutions but not in water, thus giving high results for exchangeable Na. A. H. CORNFIELD.

Transport of strontium-90 in run-off. R. G. Menzel (*Science*, 1960, **131**, 499—500).—The ^{90}Sr content of rainfall and run-off samples has been determined. Only a small proportion of the ^{90}Sr in rainfall was carried away in the run-off. However, accumulation of the isotope could occur in places where the run-off material ultimately collects. T. G. MORRIS.

Behaviour of bicarbonate and ^{85}Sr in soils. R. B. Clark, G. B. Blank, V. Q. Hale and A. Wallace (*Soil Sci.*, 1960, **89**, 292—295).—Yolo sandy loam and Hacienda (calcareous) loam were equilibrated with solutions containing $NaHCO_3$, and the losses of HCO_3^- in the filtrate determined. More HCO_3^- was lost from the calcareous loam than from the other. When the soils were dried at 50° after $NaHCO_3$ treatment the Hacienda loam lost 80—90% and the Yolo soil 75—85% of the HCO_3^-. When $^{85}Sr(NO_3)_2$ was added to the soils with either $NaHCO_3$ or NaCl the amount of ^{85}Sr remaining in solution after equilibration was the sum of the exchangeable ^{85}Sr and that precipitated as carbonate. Increased $NaHCO_3$ levels decreased the sol. Sr in both soils. Carrier Sr in general increased the sol. ^{85}Sr with both Na salts. Generally, ^{85}Sr was less sol. with Hacienda than with Yolo soil, particularly with no amendments and low HCO_3^-. T. G. MORRIS.

Ratio of caesium-137 and strontium-90 radioactivity in the soil. P. F. Gustafson (*Science*, 1959, **130**, 1404—1405).—In nine samples of soil the average value of the ratio $^{137}Cs/^{90}Sr$ was 1·62 ± 0·34. This figure is in fair agreement with that of other workers in other media. It is suggested that the ^{90}Sr content of a soil can be measured to within 20% by measuring the ^{137}Cs level and dividing by 1·6. T. G. MORRIS.

Use of spectrographic methods for total analysis of soils. M. S. Wang (*Dissert. Abstr.*, 1960, **20**, 3044).—The following method is recommended. The soil sample (100 g.) is fused with 2 g. of $Li_2B_4O_7$–GeO_2 (1000 : 1) in a Pt crucible. When cool, the glass-like product is ground, and a 200-mg. sample is mixed with 600 mg. of graphite. The mixture is briquetted at 6000 p.s.i. A centre hole is made as in the rotating disc electrode, and the sample is excited by uni-arc. Ge lines are used as internal standard lines to determine Si, Al, Fe, Ca, Mg, Mn, Ti, Zr, Sr and Ba. A. M. SPRATT.

Polyphenols in plant, humus and soil. I. Polyphenols of leaves, litter and superficial humus from mull and mor sites. II. Reduction and transport by polyphenols of iron in model soil columns. C. B. Coulson, R. I. Davies and D. A. Lewis (*J. Soil Sci.*, 1960, **11**, 20—29, 30—44).—I. Paper chromatography and electrophoresis methods were used to indicate the type and quantity of polyphenols present in fresh green and dried leaves, leaf litter and the superficial humus from mull and mor sites. The rôle of polyphenols in mull and mor formation is discussed.

II. The polyphenols, *d*- and epi-catechin, known to be present in fresh green leaves of a no. of tree species, were capable of converting the Fe of hydrated Fe_2O_3 into a sol. org. Fe^{3+} complex. The polyphenol solutions and extracts of fresh green beech leaves dissolved the Fe^{3+} in a model soil column (alumina + diatomaceous earth) treated with $FeCl_3$ and produced a dark band at lower levels in the columns. Extracts of green leaves from a mor site were more effective than those from a mull site. Extracts of humus and litter were low in polyphenols and in reducing and Fe-complexing substances. A. H. CORNFIELD.

Changes in leaf litter when placed on the surface of soils with contrasting humus types. I. Losses in dry weight of oak and ash leaf litter. K. L. Bocock, O. Gilbert, C. K. Capstick, D. C. Twinn, J. S. Waid and M. J. Woodman. **II. Changes in the nitrogen content of oak and leaf litter.** O. Gilbert and K. L. Bocock (*J. Soil Sci.*, 1960, **11**, 1—9, 10—19).—I. Oak leaves contained in nylon nets placed on the soil surface lost dry matter at about the same rate over 14 months on a mull (pH 5·6—6·3) and a moder (pH 3·2—4·7) site. Ash leaves lost dry matter at a greater rate than did oak leaves at both sites, the loss from ash leaves being greater on the mull than on the moder.

II. The N% in the ash litter increased with time on the moder but remained fairly constant on the mull site. The N% of oak litter increased on both sites, the rate of increase being greater on the moder than on the mull. Oak litter on the moder site showed an absolute increase in total N of 60% over 10 months. Possible sources of this increase are discussed. A. H. CORNFIELD.

Rates of decomposition of shortleaf pine sawdust in soil at various levels of nitrogen and lime. F. E. Allison and R. G. Cover (*Soil Sci.*, 1960, **89**, 194—201).—Slightly acid soils incubated at 30° with 1% of shortleaf pine sawdust, P, K and Mg fertiliser being added. Sawdust with enough NH_4NO_3 added to raise the N content to 2% decomposed at almost the same rate whatever its particle size

(up to 6 mesh). Sawdust mixed with soil decomposed about as quickly as that left on the surface. Addition of N increased the rate of evolution of CO_2 and lime further increased this. The rate of decomposition of sawdust varied with the ability of the soil to supply N; NO_3^- favoured decomposition more than did NH_3 unless acidity was controlled. In the same period, more C was oxidised from wheat straw than from sawdust. T. G. MORRIS.

Chemical and physical changes occurring during formation of peat from plant materials. A. F. MacKenzie (*Dissert. Abstr.*, 1960, **20,** 2983—2984).—A suggested classification for soils includes: (i) those containing predominantly identifiable plant material; (ii) those in which this material is physically disintegrated; (iii) those in which it is also chemically disintegrated; and (iv) those containing predominantly inorg. material. Cation-exchange capacity increased with increased oxidation of org. material, type (iii) soils forming a distinct group with values of 240 mequiv. or more per 100 g. Types (i) and (ii) gave similar results when submitted to column electrophoresis, but (iii) showed different distributions of org. matter and brown material. Solubility of brown material in 0·25N-acetic acid reliably indicated type (iii) soils. When examined in thin sections, type (ii) soils were physically more like (iii) than (i). Dry colour values of soils were of only limited use in determining content of inorg. matter, determination of loss on ignition being also necessary to confirm a type (iv) soil. M. D. ANDERSON.

Behaviour of free amino-acids in soil. E. L. Schmidt, H. D. Putman and E. A. Paul (*Proc. Soil Sci. Soc. Amer.*, 1960, **24,** 107—109).—Even after 1 hr. contact with soil at 4° there was considerable degradation of all of the 12 amino-acids added. After 24 hr. in soil at 28° 0—26% of the added amino-acids were left, whilst after 72 hr. nil or trace amounts only could be detected. β-Alanine was detected even though it was not added. The persistence of threonine in soil reported in the literature was confirmed. A. H. CORNFIELD.

Effects of 2,4-D on certain physiological aspects of soil micro-organisms. G. Lenhard (*S. Afr. J. agric. Sci.*, 1959, **2,** 487—497).—Microbiological activity in soil decreased with increasing applications of 2,4-D, but the microflora of the soil was not seriously affected with 2,4-D concn. <100 p.p.m. Minor amounts of 2,4-D may become toxic, due to concentration in hot dry spells after spraying. (10 references.) I. DICKINSON.

Fertiliser trials on peasant farms in Ghana. D. Stephens (*Empire J. exp. Agric.*, 1960, **54,** 1—15).—Effects of fertilisers, mainly superphosphate and $(NH_4)_2SO_4$, on groundnuts, millet, guinea corn, yams, rice and cassava in a number of localities are recorded and appropriate recommendations are made. M. LONG.

Nitrate fertilisation by ion exchange. E. Poulsen (*Physiol. Plant.*, 1959, **12,** 826—833).—Addition to soil of comparatively large amounts of NO_3^- adsorbed on an ion-exchange resin caused no adverse effects on plant growth and permitted full utilisation of the N. The same amount of N given as $Ca(NO_3)_2$ severely restricted growth. A. G. POLLARD.

Initial and residual effects of rock phosphate and superphosphate. E. C. Doll, H. F. Miller and J. F. Freeman (*Agron. J.*, 1960, **52,** 247—250).—Yields of maize, wheat and hay at two locations over 26—27 years tended to increase at a somewhat greater rate with application of rock phosphate (**I**) than with application of superphosphate (**II**) (4 times the amount of P was applied as **I** than as **II**). Where no P was applied during this period, yields on plots which had received **I** for 12—18 years previous to the 26—27 year period usually continued to increase somewhat, indicating a high residual effect. Residual effects of **II** were low, since yields declined with time during the period after discontinuing **I** applications. A. H. CORNFIELD.

Liquid fertilisers as sources of phosphorus for field crops. D. J. Lathwell, J. T. Cope, jun., and J. R. Webb (*Agron. J.*, 1960, **52,** 251—254).—Yields of wheat, oats, cotton, maize and lucerne were little different whether P was applied in solid form [conc. superphosphate, NH_4PO_3, $(NH_4)_2HPO_4$] or in liquid form [H_3PO_4, NH_4PO_3, $(NH_4)_2HPO_4$, ammoniated phosphoric acid] at the same rate of P. A. H. CORNFIELD.

Response of maize and soya-beans to magnesium fertilisers. J. L. Key and L. T. Kurtz (*Agron. J.*, 1960, **52,** 300).—Mg-deficiency symptoms occurred in maize growing on a loamy sand (pH 4·1) but not in soya-beans. Application of $MgSO_4$ (75—150 lb. Mg per acre) + 2000 lb. $CaCO_3$ per acre or dolomite (2 tons per acre) increased maize yields by about 60% and soya-bean yields by 10—30%. The treatments increased the Mg % in the plants but had no effect on % of oil or protein in the soya-bean seed. A. H. CORNFIELD.

Effects of straw ploughed in or composted on a three-course rotation of crops. H. D. Patterson (*J. agric. Sci.*, 1960, **54,** 222—229).—Lower crop yields were obtained after application of straw composted with NP than when it was ploughed in directly; losses in N, arising from drainage or immobilisation during composting were severe. Better yields were obtained when fertilisers were applied in the spring following an autumn ploughing-in of the straw. After 18 years some of the N immobilised by the straw might become available. Neither method of utilising straw affected yields by improvement in soil structure or gain in org. matter, but potatoes benefited from the K supplied by the straw. M. LONG.

Effect of city sewage effluent on hay production of small grain crops. A. D. Day and T. C. Tucker (*Agron. J.*, 1960, **52,** 238—239).—Application of sewage effluent from an activated sludge plant at a rate of 3 acre-ft. (containing N 200, P_2O_5 150 and K_2O 100 lb.) increased small grain hay yields by about 250—300% over application of the same amount of water. Application of water containing the same amount of chemical N, P and K increased yields to about the same extent as did the sewage effluent. A. H. CORNFIELD.

Phosphates and other fertilisers. Dungemittel-Technik A.-G. (B.P. 818,668, 12.2.58. Ger., 16.2.57).—Ground fertiliser, phosphate and Ca cyanamide are rendered more assimilable by plants by spraying the material under fluidised conditions with mineral acid (sulphuric, phosphoric and/or nitric acid). Apparatus is figured and claimed. F. R. BASFORD.

Fertiliser compositions. Fisons Ltd. (Inventors: F. R. Schofield and A. L. Whynes) (B.P. 818,322, 11.11.55).—NH_4NO_3 and KCl are admixed in aq. solution (during 3—20 min. at 85—100°), then the resulting solution, slurry, or preferably solidified and powdered mixture, is further admixed with phosphatic fertiliser and/or filler to provide a mixed fertiliser of reduced caking tendency. F. R. BASFORD.

Fertilisers coated with polymeric materials. Fisons Ltd. (Inventor: T. K. Hanson) (B.P. 815,829, 6.9.56).—Granular fertiliser is precoated with inorg. material, e.g., $CaSO_4$, then further coated with 0·1—10 wt.-% of a water-insol. polymeric (or polymer-forming) material, e.g., polymer or copolymer of styrene, vinyl chloride, vinylidene chloride, acrylonitrile, ethylene, or a fluorinated alkene, or with a condensation product of gelatin and formaldehyde (preferably from solution), to provide a fertiliser which is free from cracking tendency and which releases the active material slowly when added to the soil. F. R. BASFORD.

Plant Physiology, Nutrition and Biochemistry

Maximal photosynthetic rates in nature. J. Verduin, E. E. Whitwer and B. C. Cowell (*Science*, 1959, **130,** 268—269).—Photosynthetic rates are reported under natural conditions in lake water. Maximal rates appearing in the literature are lower than those obtained naturally, due, probably, to reduced turbulence in the medium of laboratory tests. T. G. MORRIS.

Photosynthesis by isolated chloroplasts. IX. Photosynthetic phosphorylation and CO_2 assimilation in different species. F. R. Whatley, M. B. Allen, A. V. Trebst and D. I. Arnon (*Plant Physiol.*, 1960, **35,** 188—193).—Isolated chloroplasts from leaves of spinach, sugar beet, pokeweed, sunflower and *Tetragonia expansa* had the ability to carry out cyclic photophosphorylation of either the flavin mononucleotide or the vitamin-K type, non-cyclic photophosphorylation, and CO_2 assimilation to the carbohydrate level. All species also showed a capacity for photosynthetic phosphorylation coupled with the reduction of ferricyanide. E. G. BRICKELL.

Disappearance of guard cell chloroplasts in ultra-violet-irradiated leaves. L. M. Blakely and M. Chessin (*Science*, 1959, **130,** 500—501).—Kidney bean leaves irradiated for 2 min. at 36 cm. distance with 130 μW/cm.² of 2537 A light lost the chloroplasts in the guard cells of the stomata within 24 hr. T. G. MORRIS.

Oxidative phosphorylation and glycollate oxidation by particles from spinach leaves. I. Zelitch and G. A. Barber (*Plant Physiol.*, 1960, **35,** 205—209).—Isolation of particles from green spinach which carry out oxidative phosphorylation with high P/O ratios, is described. Conditions are given for the oxidation of pyruvate, citrate, L-isocitrate, α-ketoglutarate, succinate, fumarate, L-malate and reduced diphosphopyridine nucleotide. O_2 uptake is mediated by the cytochrome system when org. acids of the Krebs cycle are the substrates, and by a flavoprotein oxidase with glycollate. E. G. BRICKELL.

Photocontrol of plant development by the simultaneous excitations of two interconvertible pigments. III. Control of seed germination and axis elongation. S. B. Hendricks, E. H. Toole, V. K. Toole and H. A. Borthwick (*Bot. Gaz.*, 1959, **121,** 1—8).—*Nemophila*

insignis, Lactuca sativa, var. Great Lakes and *Lamium amplexicaule* were studied. The responses observed were features of the continued excitation of the photomorphogenic pigment in those regions of the spectra (4000—5000 A and 6000—8000 A) where both pigment forms have appreciable absorptivities. E. G. BRICKELL.

Changes in the transpiration of wheat leaves caused by changes in the properties of the root medium. H. Rufelt (*Physiol. Plant.*, 1959, **12**, 390—399).—Transpiration in the leaves was rapidly (30 sec.) increased by raising the concn. of the nutrient solution from 10^{-3} to 10^{-2}M; it was lowered by rise of temp. A rapid increase followed by a decline occurred after additions of ethanol, heavy metal salts (Ag, Cu) or iodoacetic acid. A. G. POLLARD.

Effect of ultra-violet pretreatment on yield of mutations by *X*-rays in wheat. M. S. Swaminathan and A. T. Natarajan (*Science*, 1959, **130**, 1407—1409).—Wheat seeds, treated with u.v. (2537 A) light for 1 hr. or with *X*-rays at four levels of intensity up to 33,000 r, or both, were germinated, grown, and the second generation also grown. U.v. treatment alone did not induce any chromosome breakage, and no mutations occurred in the second generation. U.v. treatment decreased the mutation rates in seeds treated with 11,000 or 16,000 r of *X*-rays but increased that in seeds irradiated with higher *X*-ray levels. A significant reduction in the frequency of chromosome aberration occurred at all *X*-ray treatment levels following u.v. treatment. Pollen sterility was 15—20% in plants treated with 22,000 and 33,000 r of *X*-rays but u.v. treatment reduced this to 4—6%. T. G. MORRIS.

Possible rôle of pectic enzymes in abscission. R. E. Yager (*Plant Physiol.*, 1960, **35**, 157—162).—Conditions which restrict pectin methylesterase activity, e.g., low concn. of indolylacetic acid or presence of methionine, may cause abscission by favouring the accumulation of water-sol. pectin; other pectin enzymes may be involved in the production of pectin or by some other chemical pathway which ultimately causes dissolution of the middle lamella. E. G. BRICKELL.

Inhibitory effect of water on oxygen consumption by plant materials. T. Ohmura and R. W. Howell (*Plant Physiol.*, 1960, **35**, 184—188).—Adding water to maize, soya-bean and barley tissue resulted in marked decrease of O_2 uptake; carrot, oat and potato tissues were affected similarly but to a smaller degree. Substitution of O_2 for air in the gas phase partially or completely reversed the effect. E. G. BRICKELL.

Mechanism of absorption and accumulation of salts [by plants]. IV. Synergistic and antagonistic effects of anions. V. Retention and elution of absorbed salts. H. Lundegårdh (*Physiol. Plant.*, 1959, **12**, 336—341, 342—352).—IV. Both antagonistic and synergistic reactions between anions occur in the process of salt absorption by potato tissue and wheat roots. At low or relatively high. concn. PO_4^{3-} and SO_4^{2-} favour the absorption of Cl^-. NO_3^- is antagonistic to the intake of Cl^- only at higher concn.

V. Of the Cl^-, PO_4^{3-} or NO_3^- absorbed by the tissues in 30 min. approx. 30% was eluted by water in <4 hr. After a 24-hr. absorption period elution proceeded very slowly. CN^- and dinitrophenol hastened elution by eliminating the adsorptive power of the cytoplasm for anions. A. G. POLLARD.

Chelating agent and plant nutrient interactions affecting the iron nutrition of soya-beans. L. O. Tiffin, J. C. Brown and R. S. Holmes (*Proc. Soil Sci. Soc. Amer.*, 1960, **24**, 120—123).—Nutrient solution and soil culture tests using labelled Fe separated from the chelating agent by split-medium and split-root techniques showed that chelating agents (EDTA, DTPA and EDDHA) increased the absorption of Fe from soil and translocation of foliar-applied Fe. The effects were not sufficient to correct chlorosis. P in the nutrient medium or within the plant depressed the uptake and translocation of Fe by the plant. These effects of P on Fe were not overcome by chelating agents. The primary rôle of Fe chelates appears to be that of making Fe sol. and available to the root for absorption. A. H. CORNFIELD.

Absorption of iron from iron chelate by sunflower roots. L. O. Tiffin and J. C. Brown (*Science*, 1959, **130**, 274—275).—Sunflower seeds were germinated and transferred to Fe-free basic medium for 15 days after which time the plants were transferred to other portions of the same medium containing 5·5 mequiv. of FeEDDHA chelate per l. Fe decreased in the medium and increased in the exudate from cut stems. At the same time the chelating power of the medium increased indicating that Fe-free chelate remained in the medium; also the chelating power of the exudate remained very low. Addition of Fe to the used medium resulted in the formation of FeEDDHA indicating that free EDDHA was present. T. G. MORRIS.

Ammonium bicarbonate in plant nutrition. J. A. Goss (*Soil Sci.*, 1960, **89**, 296—302).—Beans were grown in nutrient solution containing the macronutrients, ^{32}P, B, Mn, Zn, Cu and Mo and Fe as FeEDTA. Differential treatments consisted of additions of NH_4Cl, NH_4HCO_3 or $NaHCO_3$ and NaOH to increase the pH to 8·1. In the aerial organs after 4 hr. the greatest ^{32}P activity was found with the $NaHCO_3$ treatments. NH_4Cl was more inhibitory than $NaHCO_3$, and NH_4HCO_3 gave the least P in these organs. In all cases the greatest activity was in the primary leaves and least in the trifoliate. In roots the greatest ^{32}P activity was associated with NH_4Cl in the nutrient. With mature beans the greatest ^{32}P activity in all aerial parts was found when $NaHCO_3$ and the least when NH_4HCO_3 was supplied. T. G. MORRIS.

Accumulation of bicarbonate by roots of different plant species. A. A. Bedri, A. Wallace and W. A. Rhoads (*Soil Sci.*, 1960, **89**, 257—263).—Various plants were grown in sand culture with nutrient media with and without added Fe and HCO_3^-. Addition of HCO_3^- to the nutrient caused an increase in the org. acid content of the roots to extents varying with the species, beans accumulating more than other species. Addition of Fe with the HCO_3^- lowered the org. acid levels. Use of ^{14}C-labelled HCO_3^- showed that the org. acid increase was due to assimilation of the HCO_3^- ion. Less ^{14}C was found in leaves and stems than in roots. Barley and avocado roots assimilated less HCO_3^- than did roots of beans, soya-beans or trifoliate orange plants. 70—80% of the ^{14}C in the roots was water sol. and 60—80% of the total activity was in the malic acid fraction. Two varieties of soya-beans were grown in media containing 0—5 p.p.m. of Fe and after two weeks subjected to ^{14}C-labelled HCO_3^-. Both varieties fixed more ^{14}C when they had been grown in nutrient with a low level of Fe and the activity was similar in both. High susceptibility to lime-induced chlorosis was associated with higher fixation of HCO_3^-. T. G. MORRIS.

Possible relationships of bicarbonate or CO_2 assimilation to cation accumulation by plant roots. K. C. Bhan, R. C. Huffaker, A. A. Bedri, R. T. Mueller, R. A. Jeffreys, R. M. Carmack, M. I. Biely and A. Wallace (*Soil Sci.*, 1960, **89**, 276—284).—Soya-beans were grown in nutrient solutions with N supplied as either NO_3^- or NH_4^+ and with and without HCO_3^- and at different pH levels. Yields (dry wt.) were higher with N as NO_3^- than as NH_4^+; the K, Ca and Mg totals were equally high for all treatments with HCO_3^-, but with no HCO_3^- they were lower with NH_4^+ than with NO_3^-. When intact roots were immersed in nutrient solutions containing ^{42}K and/or $H^{14}CO_3^-$ the plants accumulated two to four times as much K as HCO_3^-. Accumulation of K from $KHCO_3$ was not affected by lowering the pH from 8·5 to 7·5 nor by replacing part of the $KHCO_3$ with K_2SO_4 or KNO_3. Accumulation of HCO_3^- was lowered by diminishing the pH or $[HCO_3^-]$ in the nutrient. ^{22}Na was accumulated by excised roots equally from Na_2SO_4 or $NaNO_3$ at pH 7; at pH 8·4 more Na was assimilated from $NaHCO_3$. With ^{42}K and ^{45}Ca both in solution, the cation accumulation was related as much to pH as to the presence or absence of HCO_3^-. There was no consistent relationship between the ^{22}Na taken in from $NaHCO_3$ by the roots and the phospho-enolpyruvate carboxylase activity or the $^{14}CO_2$ fixed by roots from an enriched atm., or with the ^{14}C appearing from $H^{14}CO_3^-$ in the nutrient. T. G. MORRIS.

Relative importance of bicarbonate vs. carbon dioxide in reactions, including $KHCO_3$ accumulation by bush beans. R. C. Huffaker, R. B. Clark, R. T. Mueller and A. Wallace (*Soil Sci.*, 1960, **89**, 264—268).—Bush bean seedlings were exposed to solutions containing $KHCO_3$ tagged with ^{42}K and ^{14}C and aerated with CO_2-free air, varying levels of pH and HCO_3^- being maintained. With ribose-5-phosphate as substrate the ^{14}C fixed decreased markedly with increasing pH level, but with phospho-enolpyruvate (PEP) results were less conclusive since there appeared to be some CO_2 toxicity at pH 7·2. Above pH 8 the fixation of CO_2 with PEP as substrate decreased with increasing pH. Accumulations of ^{42}K and $H^{14}CO_3^-$ at the high $KHCO_3$ level were slightly lower at pH 9·3 than at 8 but there was little indication that it was related to the free CO_2 in the system. The ratio of ^{42}K to $H^{14}CO_3^-$ was little affected by pH, but decreased at low $KHCO_3$ levels at all pH ranges. HCO_3^- and not merely CO_2 is accumulated by roots. T. G. MORRIS.

An evaluation of bicarbonate-induced iron chlorosis. J. C. Brown (*Soil Sci.*, 1960, **89**, 246—247).—A review. T. G. MORRIS.

Possible involvement of dark fixation of CO_2 in lime-induced chlorosis. W. A. Rhoads and A Wallace (*Soil Sci.*, 1960, **89**, 248—256).—Beans were grown in aerated modified Hoagland nutrient solution (with Fe as FeEDTA), or in sand cultures; other plants were grown in ground $CaCO_3$ with additions of Fe. When Fe was supplied as ground red clay flower-pot the plants were chlorotic, but additions of FeEDTA remedied this. The org. acid content of the green and chlorotic beans was estimated. The total org. acid content was higher in chlorotic beans grown in $CaCO_3$ and red clay pot than in the green plants, the major difference being in the citric and malonic fractions. Excised roots from plants

grown in an Fe-deficient medium fixed $^{14}CO_2$ in darkness at a much greater rate than those from a medium containing Fe. Possible ways in which dark fixation of CO_2 may contribute to lime-induced chlorosis are discussed. T. G. MORRIS.

Carbon dioxide–bicarbonate absorption, accumulation effects on various plant metabolic reactions, and possible relations to lime-induced chlorosis. G. W. Miller (*Soil Sci.*, 1960, **89**, 241—245).—A review. T. G. MORRIS.

Bicarbonate and phosphorus effects on uptake and distribution in soya-beans of iron chelated with ethylenediamine di-o-hydroxyphenyl acetate). V. Q. Hale and A. Wallace (*Soil Sci.*, 1960, **89**, 285—287).—Soya-bean seedlings were immersed in a nutrient solution containing ^{59}Fe with and without P and with either Na_2CO_3 or $NaHCO_3$ in solution. Other tests used ^{59}FeEDDHA with $KHCO_3$ and KH_2PO_4. The presence of HCO_3^- and CO_3^{2-} decreased the ^{59}Fe levels in the roots and whole plant but had little effect on the Fe in leaves. P in combination with both anions decreased ^{59}Fe levels slightly. With no HCO_3^-, P increased Fe levels in roots with diminished translocation to leaves. A competitive inhibition of Fe accumulation from EDDHA by HCO_3^- and also by $H_2PO_4^-$ is indicated. T. G. MORRIS.

Effects of various levels of bicarbonate, phosphorus and pH on the translocation of foliar-applied iron in plants. R. C. Doney, R. L. Smith and H. W. Wiebe (*Soil Sci.*, 1960, **89**, 269—275).—Maize (chlorosis resistant) and bean (susceptible) plants were grown in nutrient solution containing 1 p.p.m. of supplemental Fe and various levels of HCO_3, P and pH. Nutrient Fe was lowered to 0·3 p.p.m. 2 days before foliar application of ^{59}Fe. Results after 24 hr. show the ^{59}Fe translocated from the treated leaf increased with rise in HCO_3^-, P or pH level in the case of beans. In maize max. ^{59}Fe translocation occurred with 0—10 mequiv. of HCO_3^- per l. in the medium, whereas with beans the range was 10—20 mequiv. per l. The effect of P on the Fe movement was marked, and there was a significant P–pH interaction for total movement of ^{59}Fe in maize, and for the percentage of the isotope in roots, lower stem and the treated leaf of beans. At low P levels the percentage of applied Fe found in the lower stem decreased with increasing pH, but increased at high P levels. T. G. MORRIS.

Behaviour of zinc sulphate as foliar applications and as soil applications in some New Jersey soils. R. F. Leyden and S. J. Toth (*Soil Sci.*, 1960, **89**, 223—228).—Foliar absorption of ^{65}Zn by soya-beans was small but there was translocation to all parts of the plant. Root application gave higher absorption. Larger applications had no effect on the foliar absorption but increased that from roots. Root application resulted in Zn distributions in the order root > leaves > stem > fruit. With tomatoes absorption was less by leaf than by root. With maize, absorption was greater from foliar than from root application. On a soil receiving 50 lb. of $ZnSO_4$ per acre the yield of tomatoes increased and their Zn content decreased as the pH changed from 5 to 7, more Zn coming from the soil at the same time. Less than 5% of added Zn was recovered in two crops, the recovery decreasing with rising pH. T. G. MORRIS.

Distribution of zinc in subterranean clover *Trifolium subterraneum*, L.) during the onset of zinc deficiency, as determined by the use of ^{65}Zn. D. S. Riceman and G. B. Jones (*Aust. J. agric. Res.*, 1960, **11**, 162—168).—Young plants were first grown in culture solutions containing ^{65}Zn and then transferred to solutions that were either Zn free or contained inactive Zn. Radioautographs showed that Zn taken into the leaves is retained there, even during subsequent Zn deficiency. S. G. AYERST.

Influence of pH on boron deficiency in beets and flax on clay soils of Caux. E. Jouis, M. T. Lecacheux and F. Cauchy (*C. R. Acad. Agric. Fr.*, 1960, **46**, 248—253).—The distribution of sound and B-deficient plants in a field of beet is examined in relation to soil pH. Sound plants occur principally in locations having pH <6·8. Differences in B contents of various parts of sound and diseased plants were max. in the leaves. In flax the differences were comparatively small. No close relationship was found between soil-B and soil-Ca. P. S. ARUP.

Effect of growth on redistribution of some mineral elements in peppermint. R. W. Rinne and R. Langston (*Plant Physiol.*, 1960, **35**, 210—215).—Radioautographic techniques were used to determine the long-term distribution patterns of ^{45}Ca, ^{35}S, ^{65}Zn, ^{36}Cl and ^{60}Co. Ca is only very slightly mobile in a downward direction, S accumulates more evenly in the mesophyll region of the leaves, Zn occurs primarily in stem tissue, petiole tissue and vein tissue, and Cl has a definite downward redistribution as growth proceeds. The areas of Co accumulation are the margins and tips of the leaves and Co is completely mobile. E. G. BRICKELL.

Lateral movement of phosphorus-32 in peppermint. R. W. Rinne and R. G. Langston (*Plant Physiol.*, 1960, **35**, 216—219).—Radioautographic techniques were used. Very little lateral movement of ^{32}P took place up to 144 hr. E. G. BRICKELL.

Rôle of cellular ribonucleic acid in the growth response of *Avena* coleoptiles to auxin. Y. Masuda (*Physiol. Plant.*, 1959, **12**, 324—335).—Experimental data presented lead to the theory that treatment of plant tissue with auxin increases the cation-absorption capacity of the ribonucleic acid present which then takes up Ca from the pectic material in the cell wall, thereby loosening it and facilitating cell elongation. (25 references.) A. G. POLLARD.

Chemical inhibitor of auxin-induced lateral root initiation on roots of *Pisum*. J. G. Torrey (*Physiol. Plant.*, 1959, **12**, 873—887).—Two ether- and ethanol-sol. substances inhibiting the activity of auxin are detected chromatographically, one from acid and the other from alkaline extracts of pea seedlings. (31 references.) A. G. POLLARD.

Influence of α-naphthylacetic acid on the morphology-regulating action of 2,3,5-tri-iodobenzoic acid (TIBA). O. Kiermayer (*Physiol. Plant.*, 1959, **12**, 841—853).—Morphological changes produced in tomato plants by TIBA are lessened by simultaneous application of IAA. A. G. POLLARD.

Inhibition of transport of indolylacetic acid by phenoxyacetic acids. E. Niedergang-Kamien and A. C. Leopold (*Physiol. Plant.*, 1959, **12**, 776—785).—Chlorinated phenoxyacetic acids inhibited the translocation of IAA through sunflower stems to extents which increased with the no. of substituting Cl atoms in the nucleus (max. effect with 3). A general parallelism between the influence of Cl on the inhibitory action of the phenoxy-acids and that on their adsorption on C is noted. A. G. POLLARD.

Indol-3-ylacetic acid as a protective substance against *X*-rays. E. Therman and M. Seppälä (*Physiol. Plant.*, 1959, **12**, 716—719).—Root-tip cells of *Narcissus tazetta*, L. were protected from injury by *X*-rays (200 r) by previously dipping in IAA solution for 4 hr., the effect increasing with the concn. of the solution (10—70 p.p.m.). A. G. POLLARD.

Indol-3-ylacetic acid metabolism. V. Effect of calcium ions on indol-3-ylacetic acid uptake and metabolism by pea roots. W. A. Andreae and M. W. H. Van Ysselstein (*Plant Physiol.*, 1960, **35**, 220—224).—The tips of intact roots incubated in indol-3-ylacetic acid (IAA) rapidly accumulated indolylacetylaspartic acid but they largely lost this ability on excision. This ability was restored if sucrose or Ca^{2+} was added to the medium and the uptake of IAA which was low in the absence of Ca^{2+} increased in its presence. It s suggested that Ca^{2+} maintains the tissues in a healthy state essential for normal uptake and that Ca^{2+} and sucrose prevent the loss of some endogenous factor. E. G. BRICKELL.

Effects of indolylacetic acid and other oxidation regulators on *in vitro* peroxidation and experimental conversion of eugenol to lignin. S. Siegel, P. Frost and F. Porto (*Plant Physiol.*, 1960, **35**, 163—167).—Indolylacetic acid (IAA) and other org. N compounds inhibit oxidations involving peroxidase (*in vitro*) and celery vascular tissue (*in situ*) the conversion of eugenol to lignin-like polymers *in situ* being especially sensitive to IAA, isonicotinylhydrazine and mescaline. Free-radical scavengers (chain breakers) are efficient inhibitors of eugenol oxidation in both systems, but quinones accelerate the oxidation and this may account for the failure of autoxidisable hydroxyindoles as inhibitors *in situ*. E. G. BRICKELL.

Production of indolylacetic acid by *Taphrina deformans* and *Dibotryon morbosum*. E. E. Crady and F. T. Wolf (*Physiol. Plant.*, 1959, **12**, 526—533).—In culture media containing tryptophan *T. deformans* (causing peach leaf curl) and *D. morbosum* (causing black knot in cherry and plum) produced IAA, tryptamine being the probable intermediate compound in each case. A. G. POLLARD.

Bio-regulatory activity and nitrogen function in organic compounds. Antioxidant properties and their physiological significance. S. M. Siegel, F. Porto and P. Frost (*Physiol. Plant.*, 1959, **12**, 727—741).—The rôle of antioxidants and O_2 toxicity in the operation of growth-regulating substances in plants is discussed in the light of new experimental data presented. (36 references.) A. G. POLLARD.

Effect of 2,4-D application on activity and composition of mitochondria from soya-beans. J. L. Key, J. B. Hanson and R. F. Bils (*Plant Physiol.*, 1960, **35**, 177—183).—2,4-D induced a growth of the mitochondria as indicated by changes in composition, by increased size and by increased phosphorylative activity. E. G. BRICKELL.

Gibberellic acid and osmotic pressure. J. van den Ende and P. Koornneef (*Nature, Lond.*, 1960, **186**, 327).—Tomato seedlings sprayed weekly for 10 weeks with the K salt of gibberellic acid grew

taller and bloomed earlier than unsprayed controls; no. of flowers and fruits were not affected; but fruits weighed less and the osmotic pressure of the expressed sap was raised. M. D. ANDERSON.

Chlorophyll content of gibberellin-treated wheat seedlings. F. T. Wolf and A. H. Haber (*Nature, Lond.*, 1960, **186,** 217—218).—Wheat seedlings grown in 3×10^{-4} gibberellic acid solution had the same content of chlorophyll at 6 days as those grown in water, but at 12 days contained about 20% less chlorophyll and also 20% less carotenoid. The ratio of chlorophyll *a* to chlorophyll *b* was not affected. Gibberellin-induced "chlorosis" in young seedlings results from dilution of chlorophyll owing to increased growth, and that in older plants from malnutrition, but not from direct effect of gibberellin on the metabolism of chlorophyll. M. D. ANDERSON.

Influence of gibberellic acid on the transaminase content of germinating barley seeds. G. Bergqvist, A.-M. Stensgård and N. Nielsen (*Physiol. Plant.*, 1959, **12,** 386—389).—Soaking freshly-harvested barley seed in aq. gibberellic acid (**I**) (1—30 p.p.m.) prior to germination increased the amounts of glutamic–oxaloacetic- and of glutamic–pyruvic transaminases. The possible use of this effect in determining **I** is noted. A. G. POLLARD.

Experimental separation of gibberellin and auxin actions in etiolated pea hypocotyl sections. W. K. Purves and W. S. Hillman (*Physiol. Plant.*, 1959, **12,** 786—798).—The growth response of pea seedlings to gibberellin is not actuated by any mechanism involving indolylacetic acid and no close relationship between the two growth factors is likely. (40 references.) A. G. POLLARD.

The acidic growth inhibitor of potato tubers in relation to their dormancy. M. L. Buch and O. Smith (*Physiol. Plant.*, 1959, **12,** 706—715).—Experimental evidence presented throws doubt on the theory of Hemberg (*Arkiv f. Bot.*, 1946, **33, B,** No. 2, 1) that an acid growth-inhibitor in the eye-pieces of freshly harvested potato tubers prevents immediate sprouting. A. G. POLLARD.

Action of duramycin on plant roots. A. G. Norman (*Proc. Soil Sci. Soc. Amer.*, 1960, **24,** 109—111).—The polypeptide antibiotic duramycin repressed root growth of cucumber and barley. Cells of treated roots lost both org. and inorg. solutes. The deleterious effect was mitigated in the presence of Ca^{2+} and Mg^{2+}. Duramycin did not affect auxin-induced uptake of water by hypocotyl segments. A. H. CORNFIELD.

Inhibitory effects of 2-deoxy-D-galactose on plant roots. G. Stenlid (*Physiol. Plant.*, 1959, **12,** 310—313).—Growth of seedling roots of wheat, flax, cress and cucumber was inhibited by 2-deoxy-D-galactose (**I**), the effect being reversed by glucose and galactose in cress and by glucose in wheat. In wheat absorption of Cl^- and NO_3^- was inhibited by **I** at concn. 10^{-3}M but O_2 uptake was not significantly affected at 3×10^{-2}M. A. G. POLLARD.

Coumarin and coumarin analogues as germination inhibitors of radish seeds. R. A. Bernhard (*Bot. Gaz.*, 1959, **121,** 17—21).—The seeds were germinated in presence of coumarin (**I**), 6-methylcoumarin, limettin, umbelliferone, 4-methylumbelliferone, dihydrocoumarin, *o*-coumaric acid or *trans*-cinnamic acid. Inhibitory effects were shown only by **I**, the threshold concn. for normal seedling development being in the region 10^{-3}M. Plant metabolites (octanoate, sucrose, glutathione, Ca pantothenate) in 10^{-3}M concn. did not reverse coumarin inhibition. E. G. BRICKELL.

Inhibition of photoperiodic induction by 5-fluorouracil. F. B. Salisbury and J. Bonner (*Plant Physiol.*, 1960, **35,** 173—177).—Photoperiodic induction of cocklebur is inhibited by 5-fluorouracil particularly when applied to the buds, but only fully if application is made during the inductive dark period. The inhibition is reversed by applying orotic acid. E. G. BRICKELL.

Growth inhibitor in immature soya-bean seeds and 2,4-D sprayed soya-bean seedlings. J. L. Key and D. S. Galitz (*Science*, 1959, **130,** 1340—1341).—An unidentified water-sol. growth inhibitor was found in perchloric extracts of immature soya-bean seeds in amounts 2—3 times that in mature seeds. Immature seeds leached for 3 hr. lost over half their content of the material. The compound isolated from the immature seeds or from frozen peas delayed germination of mature soya-bean seeds by 24 hr. Application of the compound to root tips increased the respiration rate for the first 10 min. but after 1 hr. the rate had declined to that of controls. In tests with mitochondria phosphate esterification was inhibited and oxidation of α-ketoglutarate was generally depressed. T. G. MORRIS.

Winter temperatures in relation to dormancy and the auxin and growth-inhibitor content of peach buds. K. L. J. Blommaert (*S. Afr. J. agric. Sci.*, 1959, **2,** 507—514).—Changes in the auxin and the inhibitor content of buds from peach trees subjected to a cold and a warm dormant period were compared. As dormancy was broken, the amount of acid inhibitor in the buds decreased more rapidly in the trees subjected to a cold dormant period. A disturbance of the auxin/inhibitor balance as a result of insufficient cold could account for delayed foliation of peach trees. I. DICKINSON.

Wax substrates in root-penetration studies. H. M. Taylor and H. R. Gardner (*Proc. Soil Sci. Soc. Amer.*, 1960, **24,** 79—81).—The use of wax substrates for simulating pressure pans in soil compaction studies is discussed and evaluated. Physical characteristics of a number of waxes are presented. The ability of plant roots to penetrate wax substrates depended upon wax rigidity, type of plant, and the density of the soil above the substrate. A. H. CORNFIELD.

Ion-exchange resin method for the fractionation of alcoholic plant extracts. D. Wang (*Nature, Lond.*, 1960, **186,** 326—327).—Recovery of 85 to 100% of ^{14}C fed to detached wheat leaves was achieved by passing alcoholic extracts (less $CHCl_3$-sol. substances) successively through columns of the ion-exchange resins Dowex-50 and AG-1. Basic substances (chiefly amino-acids) were eluted from the Dowex-50 by HCl, and acidic substances (chiefly non-volatile org. acids) from the AG-1 by formic acid. Non-ionic substances (chiefly sugars) were flushed from the AG-1 by water before eluting the acidic substances. M. D. ANDERSON.

Crops and Cropping

Effect of nitrogen on yield and quality of wheat. E. Primost (*Fertiliser News*, 1960, **5,** No. 2, 12—13).—Application of P and K fertiliser (in high doses) to winter wheat was made before sowing. N-fertiliser (Nitramoncal) (80—120 kg./ha.) was applied in early spring, at tillering and at ear emergence. Delayed N fertilisation increased yield and gluten content of the flour; the effect depended on the variety of wheat, soil and climate. Effects of increased dosage and of split applications of N on gluten content of wheat on two soils, and in two climates, together with the economical aspects are considered. E. M. J.

Nitrogen utilisation in high- and low-protein wheat varieties. J. Seth, T. T. Hebert and G. K. Middleton (*Agron. J.*, 1960, **52,** 207—209).—There were no differences in % of total N in tops or roots between low- and high-protein wheat varieties up to heading. The N % in the heads increased to a greater extent up to maturity in the high- than in the low-protein varieties. Varietal differences in N% in kernels were not much affected by soil application of NH_4^+ or NO_3^- or foliage sprays of NO_3^- or urea before heading. Foliage sprays of NO_3^- or urea applied after heading increased N% in grain in the high-protein varieties to a greater extent than that in low-protein varieties. A. H. CORNFIELD.

Establishment studies. I. Effect on spring oats of undersowing with a one-year ley. Allen H. Charles (*J. agric. Sci.*, 1960, **54,** 179—187).—Undersowing with Italian rye-grass (**I**) reduced the wt., but not the height of oat shoots, when N was not applied. When N was applied, the reduction in wt. was intensified and height was also reduced. Undersowing with red clover (**II**) affected wt. less and has no effect on height. The no. of panicles was reduced except where **I** was used without added N. **II** increased the no. of whorls and of single-grain spikelets per panicle. The no. of grains per panicle, 1000-grain wt., % of husk and of saleable grain were unaffected by undersowing, but straw yield and % of crude protein in the straw were reduced by **I** when N was applied. The moisture (%) in the sheaf was greater when the crop was undersown, especially in the case of **II**. M. LONG.

Responses of oat plants to various percentages of continuous shade. S. C. Wiggans (*Bot. Gaz.*, 1959, **121,** 55—60).—Varieties Andrew and Cherokee were studied. The no. of days from planting to maturity increased with the % of normal daylight shaded out, but there were associated decreases in plant height, no. of head-bearing tillers, and in dry wt. of grain and straw. 100-kernel wt. was not affected. E. G. BRICKELL.

Effects of soil-moisture stress at different stages of growth on the development and yield of maize. O. T. Denmead and R. H. Shaw (*Agron. J.*, 1960, **52,** 272—274).—In pot tests with maize reductions in grain yield due to moisture stress in the vegetative, silking, and ear stages were respectively 25, 50 and 21%. A. H. CORNFIELD.

Nutritional value of maize in relation to soil fertility. H. J. H. de Muelenaere and G. V. Quicke (*S. Afr. J. agric. Sci.*, 1959, **2,** 515—526).—Maize was grown under three different conditions: (i) without any fertiliser or manure for 8 years, (ii) with application of 10 tons of manure annually, and (iii) with NPK fertiliser and lime annually. No marked differences were observed in amino-acid contents between grain samples, except that the zein content

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of the maize from fertilised and manured soils was higher than that from the unfertilised soil. The protein of sample (i) was found to be superior to that of (ii) or (iii) in respect of biological value, true digestibility and net protein utilisation. (36 references.)
I. DICKINSON.

Effect of row spacing and plant population on performance of maize, grain sorghum and forage sorghum. F. C. Stickler and H. H. Laude *Agron. J.*, 1960, **52,** 275—277).—Grain and stover yields of maize were similar with 10,450—15,700 plants per acre in 20—24 in. rows. Grain sorghum yields were significantly higher with 78,000 than with 52,000 plants per acre when row spacing was 10—20 in. but not when spacing was 24—40 in. Relative evaporative power of the air within the crop was not affected by row spacing or plant population in the case of maize, but increased with row spacing in the case of sorghum. Yields of Atlas sorghum grown for silage were similar with 25,000—50,000 plants per acre and with row spacing of 20—40 in. A. H. CORNFIELD.

Potash nutrition of the potato. F. E. G. Harrap (*J. Sci. Fd Agric.*, 1960, **11,** 293—298).—The K requirement of potatoes, the relationship between rate and source of K applied and carbohydrate metabolism, K nutrition and potato quality (texture and after-cooking colour) are discussed. An optimum rate of K application (200 lb. of K_2O/acre as K_2SO_4) is suggested. (29 references.) E. M. J.

Effect of gibberellic acid on leaf area and dry-matter production in the Majestic potato. E. C. Humphries and S. A. W. French (*Ann. appl. Biol.*, 1960, **48,** 177—188).—Spraying aq, gibberellic acid (50 p.p.m.) 2 or 6 times at weekly intervals on potato plants with low or high N supply did not affect rate of leaf production on the main axis, but caused earlier senescence of leaves, especially with the more frequent spraying, and inhibited leaf production and growth on laterals of the high-N plants at nodes 10 and 11 but not at other nodes. The treatments increased leaf area even when lack of N was restricting growth, but did not produce extra dry matter. Tuber wt. was increased more on high-N plants by 2 than by 6 sprayings. The net assimilation rate of low-N plants was halved by spraying but was not changed in high-N plants, where the value was similar to that of low-N control plants. The treatments increased total and tuber dry wt. The N content of leaves per unit area was reduced by the treatments. A. H. CORNFIELD.

Quantitative changes of growth-promoting and -inhibiting substances in potato tubers treated with Rundite. I. Szalai (*Physiol. Plant.*, 1959, **12,** 237—244).—Treatment of the tubers with Rundite (ethylene chlorohydrin–ethylene dichloride–CCl_4, 7 : 3 : 1) was followed by a rapid increase in growth-promoting substance (which in the apical portions decreased on the 15th day as shoots developed) and a decrease in inhibitory substance. In non-apical parts, in which sprouting was slower, the IAA content continued to rise. Inhibitory substances in all parts of treated tubers began to increase again on the 29th day. (26 references.) A. G. POLLARD.

Surface preparation of soil for beet culture. Facts known and unknown. L. Decoux (*C. R. Acad. Agric. Fr.*, 1960, **46,** 224—247). —A review with 68 references. P. S. ARUP.

Acceleration of early growth of sugar beet seedlings by coating the seed-balls with hydrophilic colloids and nutrients. T. Miyamoto and S. T. Dexter (*Agron. J.*, 1960, **52,** 269—271).—Treatment of sugar beet seed-balls with hydrophilic colloids (Keltex, agar or gelatin) accelerated seedling emergence considerably but increased seedling size only slightly. Treatment of the seed-balls with inorg. and org. nutrients in addition to the hydrophilic coating accelerated growth of seedlings. The most effective treatment was 0·75% Keltex in Hoagland's nutrient solution without $Ca(NO_3)_2$ (5 times normal concn.) + 1% sucrose + 0·05% asparagine. Keltex treatment of seed-balls prior to storage improved germination after one year of storage. A. H. CORNFIELD.

Utilisation of fertiliser by six pasture grasses. R. W. Duell (*Agron. J.*, 1960, **52,** 277—279).—Yields of all six species of pasture grasses were increased considerably in the first two cuttings, but not in later cuttings by application of a 10—10—10 (N–P_2O_5–K_2O) fertiliser (200—1000 lb. per acre) in the spring. Yields increased with rate of application of the fertiliser, the greatest increases occurring with orchard-grass and Kentucky bluegrass. Yields of Kentucky bluegrass were usually lower than those of the other grasses in the first cutting, but usually higher than those of the other grasses in later cuttings. The N, P and K contents of all grasses increased with rate of application of fertiliser, particularly in the first cutting. Reed canary grass was consistently the highest and Alta fescue the lowest in N content. A. H. CORNFIELD.

Redistribution of nitrogen in grass and leguminous fodder plants during wilting and ensilage. C. J. Brady (*J. Sci. Fd Agric.*, 1960, **11,** 276—284).—A comparison is made of different methods of determining α-amino-N and peptide-N in short rotation ryegrass, clover and lucerne. Non-protein N increases markedly; α-amino-N increases during wilting and ensilage (**I**). In **I**, an unaccounted fraction may increase up to 25% of the total N. (25 references.)
E. M. J.

Rate, source, time and method of applying phosphates for lucerne and legume–grass hay and pasture. G. L. Terman, E. C. Doll and J. A. Lutz, jun. (*Agron. J.*, 1960, **52,** 261—264).—There were little differences in yields of grazed legume–grass between applications of concentrated and ordinary superphosphate, $Ca(PO_3)_2$ (**I**) and fused $Ca_3(PO_4)_2$ or in hay yields (lucerne or legume–grass) between applications of concentrated superphosphate and **I**. Forage yields were higher the first year of harvest with heavy initial broadcast applications than with smaller broadcast or top-dressed applications. Yields in following years with only the initial application usually became progressively poorer, as compared with yields with smaller annual top-dressings. A. H. CORNFIELD.

Yields and mineral composition of eight forage species grown at four levels of soil moisture. V. J. Kilmer, O. L. Bennett, V. F. Stahly and D. R. Timmons (*Agron. J.*, 1960, **52,** 282—285).—Yields of eight species of legumes and grasses increased with soil moisture level. Total uptake of major and trace elements increased with soil moisture level, but P was the only element which showed a consistently increasing concn. in the tissue with increasing moisture level. A. H. CORNFIELD.

Production and botanical composition of lucerne–grass combinations and the influence of the legume on the associated grasses. G. P. Tewari and A. R. Schmid (*Agron. J.*, 1960, **52,** 267—269).—Lucerne–grass mixtures yielded more forage with slightly higher lucerne content than the same combinations with lucerne and grasses in alternate rows 6 in. apart. When the two types were grown in alternate double or triple rows forage yields were even lower. Grass in rows 6 in. from a lucerne row had higher yields and protein content than when grown 12—24 in. from a lucerne row.
A. H. CORNFIELD.

Performance of an orchard grass–ladino clover mixture under clipping and grazing conditions. T. H. Taylor, J. B. Washko and R. E. Blaser (*Agron. J.*, 1960, **52,** 217—220).—There were no significant differences over 2 years between clipped and grazed forage yields from an orchard grass–ladino clover pasture. Yields were higher under both systems when the herbage was "harvested" when 8—10 in. high than when 4—6 in. high. The animals preferred clover to grass. The % of clover in the sward decreased, and that of grass increased considerably, from July of the first to July of the second year. Ladino clover was very susceptible to injury from drought and heaving. A. H. CORNFIELD.

Cutting management of grass–clover swards. II. The effects of close cutting with a gang mower or a reciprocating-knife mower on the yields from an established grass–clover sward. D. Reid and D. S. MacLusky (*J. agric. Sci.*, 1960, **54,** 158—165).—Swards cut with a gang mower (*G*) yielded 3·5—12·5% more herbage dry matter as well as more crude protein, than did those cut with a reciprocating-knife mower (*R*) due, probably, to the closer cut obtained with *G* which inhibited flower formation whilst promoting leaf formation. Increasing the no. of cuts from 6 to 8 caused a greater reduction in yield with *R* than with *G*. Broad-leaved weeds increased more rapidly with *G* than with *R* over a 3-year period, when N was not applied. M. LONG.

Effect of ammonium sulphate applications on the sulphur content of various grass and clover mixtures. D. J. C. Jones (*J. agric. Sci.*, 1960, **54,** 188—194).—Max. SO_4^{2-}-S and total S contents of the mixtures occurred with 6—8 cwt. per acre of $(NH_4)_2SO_4$; the amounts decreased with 12 cwt. per acre. Rye-grass mixtures generally contained more SO_4^{2-}-S than did others. No strict interpretation of the results in relation to hypocuprosis under British conditions is possible as some other factor appears to be involved.
M. LONG.

Effect of phosphorus, potassium and calcium on the growth, chemical composition and symptoms of deficiency of white clover in a sub-tropical environment. C. S. Andrew (*Aust. J. agric. Res.*, 1960, **11,** 149—161).—Varying amounts of Na phosphate, KCl and $CaCO_3$ were applied to a nutrient-deficient, very sandy soil. The effects on the growth of *Trifolium repens* were examined. Critical % for P, K and Ca were established. Visual symptoms of deficiency are discussed. (23 references.) S. G. AYERST.

Light and soil moisture beneath several companion crops as related to the establishment of lucerne and red clover. L. J. Klebesadel and D. Smith (*Bot. Gaz.*, 1959, **121,** 39—46).—Used as companion crops for the legumes winter wheat and rye reduced legume stands and hay yields; of spring-sown oats, wheat, barley and flax, the last was least competitive and gave the best establishment of legumes.

Legumes were also slightly more vigorous under spring wheat than under barley and oats and had slightly thicker stands but hay yields were slightly higher under barley. Growth of weeds was always least with those companion crops which caused the most shading and the greatest soil moisture stress.

E. G. BRICKELL.

Transpiration of lucerne determined from soil-water content changes. G. Ogata, L. A. Richards and W. R. Gardner (*Soil Sci.*, 1960, **89**,179—182).—The uptake of moisture by the roots of 3-year lucerne varied continuously at different soil depths. Transpiration rate is largely determined by the weather, especially immediately after irrigation, but it becomes increasingly limited and subsequently decreases as the soil dries out. T. G. MORRIS.

Influence of saline irrigation water on lucerne in lysimeters. II. M. S. Steyn (*S. Afr. J. agric. Sci.*, 1959, **2**, 473—485).—Saline irrigation water (0·3% sol. salts, Ca : Na 1 : 5) gave the highest lucerne hay yield and slightly increased the protein content. The Ca : Cl ratio in the lucerne remained constant at 2, even when the Cl content of the irrigation water was maintained at 0·115% Cl and the Ca content was varied appreciably. I. DICKINSON.

Factors affecting the hardiness of the apple. H. A. Rollins, jun. (*Dissert. Abstr.*, 1960, **20**, 3006—3008).—Cold injury in apples was assessed by determining the amount of electrolytes diffusing out of terminal twig tissues into water in 24 hr. A curve constructed from determinations after different cold treatments served to measure the degree of cold required to result in diffusion of 15% of the electrolytes that would have diffused out if the tissues had been completely killed. Varieties of apple differed markedly in cold hardiness, and in variation of hardiness at different dates. All varieties showed increasing hardiness in Nov.—Dec.; some varieties then decreased in hardiness, while others continued to become more hardy until the end of Jan. Factors that delayed tissue maturity lessened hardiness; pruning also lessened it temporarily.

M. D. ANDERSON.

Nitrogenous reserves of apple trees. K. Oland (*Physiol. Plant.*, 1959, **12**, 594—648).—Methods for extraction and determination of amino-acids etc. in the tissues are examined. Application of N to young trees affects their growth mainly in the following year, the N required for current growth being derived from reserves rather than from immediately-added sources. Reserve N is largely in the form of free amino-acids, notably arginine, in storage tissue. The concn. of sol. N in leaf tissue varies little during the season. (63 references.)

A. G. POLLARD.

Photoperiod control of growth and flowering of carnations. F. A. Pokorny (*Dissert. Abstr.*, 1960, **20**, 3005—3006).—Carnations grown with 8-hr. photoperiods, produced a larger no. of terminal cuttings at any one propagation date, but required a longer time to produce another crop of cuttings, than did stock plants grown with 16-hr. photoperiods. Cuttings from short-day plants rooted better than those from long-day plants, but cuttings rooted better with long days than with short. Cuttings from short-day stock plants flowered earlier, and produced more saleable blooms per sq. ft., than did cuttings from long-day stock plants. Photoperiod during the early growth of cuttings did not affect flower production, but long days during later growth increased the no. of flowers produced, and also lengthened flower stems. Short days during later growth gave the largest flowers. M. D. ANDERSON.

Relation between precipitation, temperature and the yield of soya-beans on the Agronomy South Farm, Urbana, Illinois. E. C. A. Runge and R. T. Odell (*Agron. J.*, 1960, **52**, 245—247).—Rainfall and max. daily temp. from June 25 to Sept. 20 explained 68% of the variation in soya-bean yields over 48 years. Above-normal rainfall during July (period of major vegetative growth) and from mid-Aug. to mid-Sept. (grain filling period) increased yields, but high rainfall during other periods decreased yields. Abnormally high temp. during July and August depressed yields.

A. H. CORNFIELD.

Influence of macro-nutrient balance in sand culture on vegetative growth and composition of nodulated and non-nodulated soya-beans. F. Haghiri (*Dissert. Abstr.*, 1960, **20**, 3003).—Nodulating and non-nodulating strains of soya-bean were grown in sand cultures with varying amounts of P, S, Ca, Mg and K. Analyses of the plants after 6 weeks showed increased yields of dry matter with high levels of nutrients. High P supplies increased the K, S and P contents of the plants; high S increased protein content; high K increased amino-acid and K contents, and decreased Ca, P and protein contents. Nodulation had varied effects on responses to different nutrients. M. D. ANDERSON.

Effect of fertilisers on the yield and quality of groundnuts in Sierra Leone. C. J. Piggott (*Empire J. exp. Agric.*, 1960, **54**, 59—64).—Lime and superphosphate individually increased yields but together did not produce any further increase. K_2SO_4 and $MgSO_4$ have no effect unless applied with lime and/or superphosphate; the effect of the latter is entirely dependent on its Ca content. K and Mg are without effect until sufficient Ca is available. M. LONG.

Effects of low concentrations of phenoxy-type growth regulators on early physiological shedding in Deltapine, TPSA and cotton. II. Influence on flower initiation. S. P. Johnson (*Agron. J.*, 1960, **52**, 294—295).—Soaking cotton seed in aq. naphthylacetic acid (0·01—0·10 p.p.m.) or 4-chlorophenoxyacetic acid (**I**) (0·0001—0·0100 p.p.m.) for 24 hr. prior to sowing had no effect on floral initiation. When seed soaked in **I** (0·1 p.p.m.) was stored at 4° for 7 days prior to sowing there was no emergence. The 0·01 p.p.m. level of **I** under these conditions inhibited both axillary bud and internode development and the fruiting branch appeared at higher nodes.

A. H. CORNFIELD.

Foliar abscission in cotton. III. Macroscopic and microscopic changes associated with natural and chemically induced leaf fall. C. H. Leinweber and W. C. Hall (*Bot. Gaz.*, 1959, **121**, 9—16).—Defoliants such as Endothal (disodium 3,6-endoxyhexahydrophthalate) induce and accelerate the dropping of leaves in much the manner of natural abscission, which involves secondary cell division. On the other hand defoliants such as Shed-A-Leaf (sodium chlorate–metaborate) and 3-amino-1,2,4-triazole, stimulate a rapid hydrolysis of walls across the abscission zone with little or no cell division preceding separation. E. G. BRICKELL.

Manuring of cotton in West Pakistan. II. Effect of date of sowing, irrigation, spacing between rows and rate of manuring on yield of seed cotton. A. Wahhab and R. Ahmad (*Empire J. exp. Agric.*, 1960, **28**, 35—43).—Best sowing dates ranged from early May to early June, according to area. The highest yields were obtained with the earliest sowing dates and 6 irrigations. Close spacing (2 ft.) was relatively better in conditions of limited water supply. Two levels of $(NH_4)_2SO_4$ (50 and 75 lb. of N/acre) were both effective. The N effect decreased in later sowings with wider spacing and increasing irrigation. N applications were economic only with early-sown crops. M. LONG.

Manuring of cotton in West Pakistan. III. Effect of rate and kind of manuring and date of sowing on the yield of seed cotton. A. Wahhab and R. Ahmad (*Empire J. exp. Agric.*, 1960, **54**, 64—73).—$(NH_4)_2SO_4$ was superior to farmyard manure (FYM) either alone or mixed with some $(NH_4)_2SO_4$, on early-sown crops but FYM is more effective on late-sown crops. High day temp., low night minima, and high R.H. favoured the crop; early sowing was especially beneficial. M. LONG.

Nitrogen, phosphorus and potassium requirements of sugar cane. R. F. Innes (*J. Sci. Fd Agric.*, 1960, **11**, 299—309).—Responses of sugar cane to fertilisers in Jamaica and in other sugar-producing countries are surveyed. Soil analyses, deficiency symptoms and tissue analyses are considered in relation to the nutritional status of the plant. (35 references) E. M. J.

Manuring of green crops used in tea culture. S. K. Dutta and K. N. Sharma (*Empire J. exp. Agric.*, 1960, **54**, 53—58).—Growth of *Crotalaria anagyroides* on land recently cleared of tea was increased by application of superphosphate (P_2O_5 80 lb./acre) especially in the earlier stages. An additional dressing of K increased the yield of seed but had no further effect on total dry wt. of the crop.

M. LONG.

Nutrition of forest tree seedlings. II. Mineral nutrition of spruce. T. Ingestad (*Physiol. Plant.*, 1959, **12**, 568—593).—Spruce seedlings were grown in nutrient solutions and threshold levels of visual deficiency symptoms (chlorosis in most cases) in needles and roots are determined in respect of N, P, K, Ca, Mg, Fe and S. The absolute requirements as measured by the total amount present in needles in max. growth were in the descending order, N, K, P, Mg, Ca and were less than the corresponding values for birch or pine. (70 references.) A. G. POLLARD.

Seasonal changes in white pine leaves: a comparison of cold resistance and free-sugar fluctuations. J. Parker (*Bot. Gaz.*, 1959, **121**, 46—50).—Eastern white-pine leaves increased in hardiness in autumn by a series of stages, apparently partially controlled by low (daily min.) temp. By Dec. all trees were resistant to at least −78° and in late winter to at least −196° provided the rate of cooling and warming was less than 10° per hr. Raffinose was closely associated with hardiness and increased up to about 1·5% of the fresh wt. Fructose and glucose did not show such clear relationships and sucrose retained a high level in spring in spite of dehardening. E. G. BRICKELL.

Effect of salt concentration on growth of red mangrove in culture. W. L. Stern and G. K. Voigt (*Bot. Gaz.*, 1959, **121**, 36—39).—Seedlings of *Rhizophora mangle* in sand culture were supplied with solutions containing K_2SO_4 and chlorides of Na, Mg and Ca in constant

proportions but in varying total concn. The salt solutions increased dry matter production especially at the concn. approximating to that of sea water. Early development is favoured by a high salt concn. E. G. BRICKELL.

Limitation to growth of basswood from mineral nutrient deficiencies. W. C. Ashby (*Bot. Gaz.*, 1959, **121**, 22—28).—Lower total dry wt. and visual symptoms of deficiency were established in *Tilia americana*, L. due to deficiencies of N, P, K, Ca or Mg in sand culture; S deficiency appeared to be without effect. E. G. BRICKELL.

Sulphur deficiency in *Tilia americana*. W. C. Ashby and E. S. Mika (*Bot. Gaz.*, 1959, **121**, 28—31; cf. preceding abstr.).—A closed chamber technique showed that growth was limited by S deficiency; visual symptoms included yellowed leaves, early death of the growing point leading to diminished height, leaf no. at harvest, and dry wt. Other than smaller size, no visual symptoms of root damage were noted. E. G. BRICKELL.

Toxic principle in the leaves of ailanthus. F. Mergen (*Bot. Gaz.*, 1959, **121**, 32—36).—The presence of a substance toxic to other tree seedlings was demonstrated in aq. extracts of foliage from *Ailanthus altissima*, (Mills) Swingle. Of 35 species of gymnosperms and 11 species of angiosperms tested with the extract all, with the exception of *Fraxinus americana*, L., were adversely affected. E. G. BRICKELL.

Compositions for controlling plant growth. Shell Research Ltd. (Inventors: G. E. Barnsley, J. K. Eaton and R. S. Airs) (B.P. 818,925, 16.5.55).—An org. B compound, viz., phenyl or diphenylborinic acid substituted by halogen, Me, halogenomethyl- or OMe, unsubstituted diphenylborinic acid, or an anhydride, an ester or a salt thereof (with a N-base), is compounded with a liquid or solid carrier and a surface-active agent, to provide a composition for use in controlling the growth of plants. Thus an aq. suspension containing Triton X-100 (0·05) and *o*-$ClC_6H_4 \cdot B(OH)_2$ (0·1%) is 100% lethal to mustard seedlings (rate of application, 5 lb. per acre). F. R. BASFORD.

Pest Control

New series of organic compounds having polyvalent fungicidal activity. G. Rossi and V. Corradini (*Chim. e Industr.*, 1960, **42**, 237—242).—Acetates and benzoates of various dialkyldithiocarbamoyl and alkylxanthyl-carbinols are prepared. Of these, dimethyldithiocarbamoylcarbinol benzoate is particularly effective against oidium, mildew and rust. (10 references.) J.A.C. ABSTR.

New fungicide active against *Botrytis* species. N. G. Clark, A. F. Hams, D. J. Higgons and H. A. Stevenson (*Chem. & Ind.*, 1960, p. 572—573).—The compound 2,6-dichloro-4-nitroaniline (**I**) is fungistatic towards *Botrytis* spp., non-toxic to a wide range of plants, and non-irritant to users; its chemical stability and low solubility and volatility render it particularly persistent in action. Better results were obtained on lettuce and other crops with **I** than with other commonly used compounds. Control of potato-rot was also obtained. P. S. ARUP.

Penetration and stability of GS-1 in plant tissue. J. Dekker and P. A. Ark (*Physiol. Plant.*, 1959, **12**, 888—892).—The fungicidal action of GS-1 [2-(1-oxy-2-pyridylthio)imidazoline hydrobromide] on various plant pathogens is demonstrated. It acts systemically in some plant species but is inactivated in others. A. G. POLLARD.

Distribution and sorption of liquid fumigants applied to wheat by recirculation. W. K. Whitney and E. E. Kenaga (*J. econ. Ent.*, 1960, **53**, 259—261).—Serafume, a mixture of CCl_4 76·5, ethylene dibromide 3·5, CS_2 10 and ethylene dichloride 10 wt.-% was circulated in 6-ft. bins. The concn. of the components at different times and different levels is given. Samples were analysed by a thermal conductivity analyser and a mass spectrometer. Sorption rates are also given. C. M. HARDWICK.

Fungicidal effectiveness of *S,S*-dimethyl xanthogen-ethylenebis(dithiocarbamate) and related compounds. D. C. Torgeson, J. A. Lambrech and W. H. Hensley (*Contr. Boyce Thompson Inst.*, 1960, **20**, 299—305).—Inhibition of spore germination and control of early and late blight of tomatoes were used as assay methods. The Me, Et, Pr^i and Bu^n homologues were about equally fungitoxic but toxicity was reduced when the alkyl group was increased to cyclohexyl or 2-ethylhexyl and when the alkylene group was increased above ethylene. A disulphide linkage was more active than the tri- or tetrasulphide. *S,S'*-Dimethyl xanthogen-ethylenebis(dithiocarbamate) was found to be as effective as zineb for the control of potato late blight. (12 references.) E. G. BRICKELL.

Stability, toxicity and reaction mechanism with esterases of certain carbamate insecticides. J. E. Casida, K. B. Augustinsson and G. Jonsson (*J. econ. Ent.*, 1960, **53**, 205—212).—Various *N*-alkyl-and *NN*-dialkylcarbamates were compared as inhibitors of acetyl- and butyryl-cholinesterase and for toxicity to *Musca domestica*. The configurations significant biologically were not related to stability. Competition rather than carbamoylation is indicated as the primary mechanism of cholinesterase inhibition.(33 references.) C. M. HARDWICK.

Epoxidation of aldrin and heptachlor in soils as influenced by autoclaving moisture and soil types. E. P. Lichtenstein and K. R. Schulz (*J. econ. Ent.*, 1960, **53**, 192—197).—Under conditions favourable to micro-organisms aldrin added to soil was converted into dieldrin almost quant. in 3—16 months. Heptachlor did not readily change to its epoxide. (12 references.) C. M. HARDWICK.

Laboratory selection of normal and chlordane-resistant German cockroaches for resistance to malathion and diazinon. J. McD. Grayson (*J. econ. Ent.*, 1960, **53**, 200—203).—Chlordane-resistant *Blattella germanica* were more resistant to malathion than were normal ones but when selection pressures of >50%, >65% and >80% were applied to the subsequent parent generations, malathion resistance developed more rapidly in the chlordane-resistant strains. (11 references.) C. M. HARDWICK.

Boll weevil susceptibility to toxaphene, endrin and Guthion in five Alabama localities. G. F. Burkhalter and F. S. Arant (*J. econ. Ent.*, 1960, **53**, 311—313).—Topical application to 2-day-old weevils showed no great variation in susceptibility in the different regions; variation during and between two years was of the same order. C. M. HARDWICK.

Effect of relative humidity on hatching and on toxicity of ovicides to eggs of red spider mite, *Tetranychus telarius*, L. R. A. Harrison and A. G. Smith (*Nature, Lond.*, 1960, **186**, 409).—Mortality of eggs of *T. telarius* decreases from ~60% at 10% R.H. to a min. of ~4% at 60% R.H. and rises steeply from 20 to 100% at >98% R.H. The LC_{50} decreases with increasing R.H. when the freshly-laid eggs are dipped in ovicides (wettable powder) and then incubated at different R.H. at 23°. Mortality-LC_{50} curves are parallel at each R.H. For Kelthane LC_{50} = 0·02% at 30% and 0·0005% at 90% R.H. W. J. BAKER.

Contribution of surface characters to wettability of leaves. S. B. Challen (*J. Pharm., Lond.*, 1960, **12**, 307—311).—A technique based on the prep. of carnauba wax positive replicas of leaf surfaces, is described. Macroscopic surface roughness is the chief factor preventing the wetting of *Festuca pratensis*. The contribution of surface chemistry and surface roughness is discussed also for leaves of *Agropyron repens* and two species of *Papaver*. E. G. BRICKELL.

Topical application of mobile liquids to insects by means of microcapillary tubes. P. S. Hewlett and C. J. Lloyd (*Ann. appl. Biol.*, 1960, **48**, 125—133).—A self-filling micro-pipette, capable of delivering reproducible doses of 0·03 μl. upwards on insects, is described. Equipment for keeping insects under anaesthesia during their dosage is also described. A. H. CORNFIELD.

Evaluation of stable fly toxicants and repellants. R. H. Roberts, C. M. Jones and E. E. Gless (*J. econ. Ent.*, 1960, **53**, 301—303).—For preliminary evaluation *Stomoxys calcitrans*, exposed in cages to 6-in.-diameter treated areas on a cow, gave a fair indication of the residual effectiveness and repellancy of an insecticide. Secondary evaluation was by cage tests in which flies were liberated with a yearling previously sprayed with 1 gal. of test solution. C. M. HARDWICK.

Use of Phorate to control aphids and the hessian fly on winter wheat. M. C. Wilson, H. F. Hodges, R. L. Gallun and R. E. Kirk (*J. econ. Ent.*, 1960, **53**, 197—200).—Seed treatments of 0·5 and 0·75 lb./100 lb. wheat gave complete control of *Phytophaga destructor*, and *Rhopalosiphum fitchii* and *Macrosiphum granarium*. The spring infestation was controlled by a broadcast application of 1·75 lb./acre of Phorate granules. Increased yields were obtained from both hessian fly-susceptible and -resistant varieties in spite of germination and stand reductions of up to 50%. C. M. HARDWICK.

Greenbug control with systemic insecticides as influenced by fertiliser applications. N. E. Daniels (*J. econ. Ent.*, 1960, **53**, 278—279).—Addition of demeton to a fertiliser application lengthened the period of protection against *Toxoptera graminum* for potted wheat plants by 18 days. Soil treatment was more effective than seed treatment. C. M. HARDWICK.

***Hyperodes humilis*, a new pest of sweet maize in the Everglades and its control.** E. D. Harris, jun. (*J. econ. Ent.*, 1960, **53**, 251—257).—The life history, host plants and damage are described. Pre-planting treatments of soil with heptachlor, parathion or a mixture

did not reduce the attack of *H. humilis*. DDT at 2 lb./100 gal. or Guthion at 1 lb./100 gal. applied at 4-day intervals to stem and soil for a month gave good control. (12 references.)
C. M. HARDWICK.

Effectiveness of mixtures of acrylonitrile and carbon tetrachloride against three pests of stored maize. R. F. Ruppel, G. Bravo and W. H. Hatheway (*J. econ. Ent.*, 1960, **53**, 238—242).—Maize, heavily infested with *Sitophilus oryza*, *Sitotraga cerealella* and *Tribolium confusum* was fumigated with 1 : 2, 1 : 4, 1 : 6 and 1 : 8 mixtures of acrylonitrile–CCl_4 at concn. of 0·05, 0·1 and 0·2 ml./3 l. of maize for 24 hr. A 3 : 1 mixture of $C_3H_4Cl_2$ and CCl_4 at 0·4 ml./3 l. was used for comparison. The two highest dosages of the 1 : 2 mixture and the highest dosage of the other mixtures were effective against all three spp. after 24 hr., but after 6 weeks only the highest dosage of the 1 : 2 mixture and the standard were satisfactory. The interaction of the species and their reaction to the mixtures is discussed. (13 references.) C. M. HARDWICK.

Susceptibility of certain stages of the rice weevil to a methallyl chloride fumigant formulation in wheat of various moisture contents. J. R. Pedersen (*J. econ. Ent.*, 1960, **53**, 288—291).—Wheat with 11, 12 and 14% moisture content was fumigated with a 80 : 20 mixture of CCl_4 : methallyl chloride for 48 hr. at 80°F in gallon jars. The effect on eggs, first and third instar larvae, pupae and adult *Sitophilus oryza* was recorded. Mortality due to fumigation was complete within 5 days. The amount of fumigant needed increased with increasing moisture content. LC_{50} and LC_{90} for the different stages are given. (13 references.) C. M. HARDWICK.

Residues in established lucerne treated with granulated Phorate (Thimet) and their effect on cattle fed the hay. R. C. Dobson, G. O. Throneberry and T. E. Belling (*J. econ. Ent.*, 1960, **53**, 306—310).—Residues from a 1 lb./acre application of granulated Phorate were not detectable after 14 days. With 4 lb./acre residues were high at 21 days and detectable at 25 days. Analysis was by an electrometric method based on inhibition of human plasma cholinesterase. No general reduction in blood-cholinesterase was found.
C. M. HARDWICK.

Control of army cutworms in lucerne during 1959. R. E. Pfadt (*J. econ. Ent.*, 1960, **53**, 319—320).—Sprays of SD 4402 (1,3,4,5,6,-7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanophthalan), Endrin and Thiodan gave effective control of *Chorizagrotis auxiliaris*. Granulated endrin and dieldrin spray were ineffective.
C. M. HARDWICK.

Control of woolly aphid on roots of apple nursery stock. E. O. Hamstead and E. Gould (*J. econ. Ent.*, 1960, **53**, 217—220).—Good reduction in numbers of *Eriosoma lanigerum* was obtained by spraying soil round the trees with BHC, lindane or aldrin. Paste and activated C dips prior to planting were not satisfactory. Trunk sprays of BHC, Sevin, demeton and Guthion gave good results but Trithion, schradan and Nemagon were less effective.
C. M. HARDWICK.

Control of pear borer and American plum borer in apple trees. L. P. Kelsey and L. A. Stearns (*J. econ. Ent.*, 1960, **53**, 276—279).—Control of *Euzophora semifuneralis* and *Thamnosphecia pyri* was dependent on spraying with sufficient force to penetrate the bark. Both parathion and various BHC formulations were effective.
C. M. HARDWICK.

Thiodan, a promising chemical for control of the lesser peach tree borer. D. H. Moore (*J. econ. Ent.*, 1960, **53**, 321—322).—A simple spray of Thiodan or parathion produced 70% control of *Synanthedon pictipes*; this was increased to 93% with 3 applications.
C. M. HARDWICK.

DDT deposits on grapes as affected by growth and weathering. E. F. Taschenberg and A. W. Avens (*J. econ. Ent.*, 1960, **53**, 269—276).—Residues from a three-application regime were analysed for 7 years. Growth between the last spray and the beginning of harvest decreased the residues by ~27% and weathering by ~37%. A residue of <7 p.p.m. was obtained when only two applications were made. The influence of spray additives is also analysed. (24 references.) C. M. HARDWICK.

Effect of parathion on parasites of oriental fruit moth cocoons and *Trichogramma minutum* in peach orchards. M. H. Brunson (*J. econ. Ent.*, 1960, **53**, 304—306).—Parasitisation of cocoons pinned on peach trees was 86% lower on those sprayed with parathion than on those sprayed with Pb arsenate; the no. of species was also lower. Parasitisation of eggs by *Trichogramma minutum* was 20% less in orchards sprayed with parathion than in those treated with Pb arsenate although this level varied greatly. (11 references.)
C. M. HARDWICK.

Susceptibility of life stages of the Mexican fruit fly to fumigation with ethylene chlorobromide. C. A. Benschoter (*J. econ. Ent.*, 1960, **53**, 323—325).—In laboratory experiments the order of susceptibility at LD_{99} was larva > adult > egg > pupa. Dosage-mortality regression lines are given for each stage. C. M. HARDWICK.

Effect of soil treatments of chlordane or heptachlor on the flavour of turnips, parsnips and carrots. M. E. Kirkpatrick, J. L. Brogdon and G. J. DeViney (*J. econ. Ent.*, 1960, **53**, 285—287).—No off-flavour was found in any of the vegetables, cooked or raw, after treatment with chlordane at 5, 10 and 20 lb./acre or heptachlor at 3 and 6 lb./acre. C. M. HARDWICK.

Effect of aldrin on clubroot of summer cabbage. A. G. Channon and W. G. Keyworth (*Ann. appl. Biol.*, 1960, **48**, 1—7).—In 1956 application of aldrin (2—8 lb. per acre, or 0·125 pint of 0·04% emulsion per plant) had little effect on the incidence of clubroot in summer cabbages. In 1957 the higher rates of aldrin gave some control. Liming the soil also gave some control and best control was obtained by liming + aldrin. In 1958 control increased with the dose of aldrin per plant, but some phytotoxicity occurred with the heavy dose (0·5 pint of 0·135% aldrin emulsion).
A. H. CORNFIELD.

Incidence and enzymic activity of moulds found in Indiana and Ohio tomatoes. L. S. White (*Dissert. Abstr.*, 1960, **20**, 3026—3027).—*Alternaria solani* and *Colletotrichum phomoides* were widely distributed in field-grown tomatoes, but often produced only minor lesions. Spp. of *Oospora*, *Rhizopus*, *Fusarium* and *Mucor* were not so often found, but were the most active in producing rot. Moulds usually increased the pH of tomato juice, perhaps thereby contributing to the development of "flat sour" spoilage. Infection with moulds usually produced some off-flavour. Howard mould counts were often high when little rot was present, and a high % of visible rot may co-exist with low mould counts. Tomato tissue and juice containing active rot-producing moulds showed high polygalacturonase activity, but concn. of cellulase was not correlated with rot. M. D. ANDERSON.

Control of two-spotted spider mite on beans with systemic insecticides applied in the soil. J. Wilcox and A. F. Howland (*J. econ. Ent.*, 1960, **53**, 224—227).—Phorate and Disyston gave up to 95% control of *Tetranychus telarius* on lima beans for 2—3 months when applied to or with the seeds. Similar control is given by foliage sprays. Doses of <1 lb./acre were as effective as higher doses and less phytotoxic. Phorate (2—4 lb./acre) gave good control of *Frankliniella moultoni*. C. M. HARDWICK.

Field control of cotton mites with Aramite aerial spray in California. J. P. Corkins and N. W. Hazel (*J. econ. Ent.*, 1960, **53**, 203—205).—Comparative tests with various oil solutions and emulsions of Aramite [2-(*p*-t-butylphenoxy)-1-methylethyl 2-chloroethyl sulphite] are recorded. C. M. HARDWICK.

Control of boll weevils resistant to chlorinated hydrocarbons. J. K. Walker, jun., and R. L. Hanna (*J. econ. Ent.*, 1960, **53**, 228—231).—Five-day applications of Guthion, Sevin and Ca arsenate all gave control of *Anthonomus grandis*. Malathion and methyl parathion needed more frequent application because of limited residual effectiveness. The effect of the addition of DDT to other chlorinated hydrocarbons and phosphates varied with the degree of local resistance. C. M. HARDWICK.

Small amounts of insecticides effective in controlling grubs of the Japanese beetle. J. B. Polivka (*J. econ. Ent.*, 1960, **53**, 320).—From previously published work the min. amount of chlordane, BHC, heptachlor and aldrin necessary for effective control of Japanese beetle in lb./acre is deduced. C. M. HARDWICK.

Laboratory evaluation of several insecticides against *Chrysops* larvae. R. A. Hoffman (*J. econ. Ent.* 1960, **53**, 262—263).—Larval mortality was compared 48 hr. after introduction into the insecticidal solutions. Parathion was most effective, giving 100% mortality at 5 p.p.m. Lindane gave 95% mortality at 10 p.p.m. All other chlorinated hydrocarbons were less effective than the org. P compounds. Thiocyanates gave poorest results.
C. M. HARDWICK.

Insecticidal baits for control of imported fire ant, *Solenopsis saevissima richteri*. S. B. Hays and F. S. Arant (*J. econ. Ent.*, 1960, **53**, 188—191).—Laboratory study of 104 baits showed that cooking oils and groundnut butter were most acceptable. Extensive mound and area treatments showed that Kepone with an oil and tankage gave the highest level of control. Heptachlor up to 0·042% was also good but at higher concn. acted as a repellent.
C. M. HARDWICK.

2,6-Dichlorobenzonitrile: new herbicide. H. Koopman and J. Daams (*Nature, Lond.*, 1960, **186**, 89—90).—Several benzonitriles inhibit the growth of young oat plants and the germination of seeds of many weed species. The most active compound tested was 2,6-dichlorobenzonitrile, its activity being 100 times that of isopropyl *N*-phenylcarbamate. C. A. SLATER.

Mode of action of dipyridyl quaternary salts as herbicides. R. F. Homer, G. C. Mees and T. E. Tomlinson (*J. Sci. Fd Agric.*, 1960, **11**, 309—315).—The herbicidal activity of certain diquaternary salts of 2,2′-, 2,4′- and 4,4′-dipyridyl depends on their redox properties, giving stable water-sol. free radicals by uptake of a single electron on reduction. A close relationship was found between the redox potential and the degree of herbicidal activity. The herbicide is reduced in green tissues by energy ultimately derived from light and the reducing system probably resembles that operating in photosynthesis. In darkness the lethal mechanism acts more slowly and is probably associated with respiration. (13 references.)
E. M. J.

Herbicide studies. I. Translocation and use of sodium 2,2-dichloropropionate as a herbicide. II. Use of herbicides in establishing legume seedlings. P. W. C. Santelmann (*Dissert. Abstr.*, 1960, **20**, 3008—3011).—I. Dalapon at 5—40 lb. per acre killed the foliage of quackgrass, but recovery always occurred from the rhizomes. Ploughing before or after the application increased the effect. Second-year red clover was very susceptible to dalapon; lucerne was less susceptible, but its growth was somewhat checked. In greenhouse experiments, 0·5% dalapon killed quackgrass foliage, but 1% was required to kill rhizomes. Dalapon was rapidly absorbed by the foliage, and translocated to other parts of the plant; it was also absorbed by the plant from the soil.

II. Tests were made of the effects of various herbicides on crops of legumes, which grow slowly at first, and may thus be overpowered by weeds. DNBP at 1 or 2 lb. per acre, applied before or just after emergence, was effective in killing weeds without harming legumes; 2,4-D at 0·25 lb. per acre, and amine salts of MCP or 2,4-D at 0·25 lb. per acre, applied soon after emergence, were also useful; esters of 2,4-D injured the legumes. TCA and dalapon were of doubtful value. M. D. Anderson.

Biochemical effects of 2,4-dichlorophenoxyacetic acid on plants. J. L. Key (*Dissert. Abstr.*, 1960, **20**, 3050—3051).—Soya-bean seedlings sprayed with 5×10^{-4}M-2,4-D showed increases in contents of protein, nucleic acid and acid-sol. nucleotide, 24—48 hr. later. Changes in composition of mitochondria and in activity of oxidative phosphorylation are recorded. Sprayed plants, especially apical tissues, contained a growth inhibitor, apparently able to uncouple phosphorylative oxidation. The compound, which is not a nucleotide, is present in high concn. in immature soya-bean seeds that will not germinate, and in low concn. in mature seeds; it disappears on germination, but reappears on spraying with inhibiting concn. of growth regulators. M. D. Anderson.

Chemical activity of the glucose adduct of 3-amino-1,2,4-triazole. A. C. Gentile and J. F. Fredrick (*Physiol. Plant.*, 1959, **12**, 862—867).—The adduct of the herbicide (3AT) with glucose, probably the amine glucoside, is metabolised within the plant much more slowly than is free glucose. The possible action of 3AT in sequestrating part of the glucose in the plant and thus disturbing its normal metabolism is suggested as the basis of the herbicidal action.
A. G. Pollard.

Vegetative and reproductive growth response of three *Trifolium* species to 2, 4-D. D. P. Ormrod and W. A. Williams (*Agron. J.*, 1960, **52**, 229—234).—Propylene glycol butyl ether ester of 2,4-D was applied at 0·3 and 3 lb. of 2,4-D-acid-equiv. per acre in 10 and 100 gal. water at various growth stages, to rose clover (**I**), *Trifolium hirtum*, All., subterranean clover, *T. subterraneum*, L. (**II**), and crimson clover, *T. incarnatum*, L. (**III**). **II** was much more tolerant to 2,4-D applied when the clovers were growing actively than were **I** and **III** as measured by forage and seed production, viability of seed from treated plants, and stand in the year following treatment. **III** was somewhat more tolerant of the low rate of 2, 4-D than was **I** when application was made at pre-bloom stage. Treatment at any bloom stage produced seed which germinated into malformed seedlings. Malformed seedlings were fewer with the high than with the low spray vol. where the low rate of application was made.
A. H. Cornfield.

Emulsifier and emulsifiable concentrate. Atlas Powder Co. (B.P. 816,915, 20.12.56. U.S., 27.12.55).—An anionic-non-ionic emulsifier composition consists of an amine (alkylenediamine or polyalkylenepolyamine of 2—8 C) salt of an alkylarylsulphonic acid (alkyl of 8—20 C of which at least 4 are in a single alkyl radical) (20—50%) and a polyoxyethylene ether of a hexitan mono-tall oil ester of 10—20 oxyethylene groups (50—80%). The composition is equally effective in hard and soft water and may be dissolved (70—75%) in an aromatic hydrocarbon (trimethylbenzene or a heavy aromatic naphtha) to provide a concentrate especially useful in the compounding of insecticides, herbicides and agricultural chemicals. A typical formulation comprises: trimethylbenzene (25), ethylenediamine dodecylbenzenesulphonate (25) and polyoxyethylene ether of sorbitan mono-tall oil ester containing 16 oxyethylene groups per mol. (50%). F. R. Basford.

Phytotoxic and insecticidal preparations and compounds. N.V. Philips' Gloeilampenfabrieken (B.P. 818,437, 14.6.57. Neth., 15.6.56).—Compounds, useful as phytotoxic agents, insecticides and fungicides comprise 4,6-dichloro-*s*-triazines substituted in the 2-position by XR (X is O or S; R is saturated or unsaturated aliphatic hydrocarbon radical or cycloalkyl of ⩽2C). The prep. of 4,6-*dichloro-2-ethoxy-s-triazine*, b.p. 114°/4 mm., in 52% yield is described. F. R. Basford.

Pyrimidine derivatives and fungicidal compositions containing them. J. R. Geigy A.-G. (B.P. 817,359, 14.2.57. Switz., 15.2.56).—Compounds useful as fungicides comprise pyrimidines substituted in the 4-position by Me or Et; in the 2-position by CCl_3·SX (X is O or S); and optionally in the 5-position by alkyl of 1—2 C (or a trimethylene or tetramethylene chain whose other end then replaces the 4-substituent) and in the 6-position by OH, halogen, Me, Et or CCl_3·S·X. They are made by treating a corresponding pyrimidine substituted in the 2-position by XM and optionally in the 6-position by XM, SH, OH, Me or Et (M is alkali metal) with CCl_3·SCl. Good yield of 6-*hydroxy-2-trichloromethanesulphenylthio-4-methylpyrimidine*, m.p. 149—150° (decomposition), is prepared. When compounded with talc it affords a pulverulent fungicide which can be successfully used for disinfecting bedding earth and for dusting bulbs, tubers and plants. F. R. Basford.

Biologically active compositions containing dihydro-oxazolones. Shell Research Ltd. (Inventors: J. T. Hackmann and P. Ten Haken) (B.P. 818,186, 20.7.56).—There are claimed compositions containing dihydro-oxazolones substituted in the 2-position by a hydrocarbon or heterocyclic radical (optionally substituted by halogen) and in the 4-position by :CRR′ (R is hydrocarbon or heterocyclic radical, optionally substituted by halogen or NO_2; R′ is H or hydrocarbon radical; or R and R′ together with C form an acyclic or a heterocyclic radical). The carrier for the active agent may be a solid, a petroleum oil (especially a fraction, b.p. 136—300° or 300—540°, containing ⩽75% of unsulphonatable residue), or other liquid, and the composition may also contain a surface-active agent. According to the nature of the substituents in the dihydro-oxazolone, the composition may be useful as a fungicide, a herbicide, a fungus (yeast, mushroom) stimulant or an anti-virus agent. Thus, application of spindle oil containing 0·1 wt.-% of 2-phenyl-4-(anthr-9′-ylmethylene)oxazol-5-one to mustard seedlings causes 96% reduction in growth, without affecting oats. F. R. Basford.

Vinyltin chlorides and bromides. Metal & Thermit Corp., Assee of S. D. Rosenberg and A. J. Gibbons, jun. (B.P. 815,954, 12.9.57. U.S., 18.9.56).—The prep. is described of SnX_aR_{4-a} where a is 1—3 and X is Clor Br, e.g., *vinyltin trichloride*, useful *inter alia* as fungicides, nematocides and slimicides. J.A.C. Abstr.

Polymeric bis-thiuram disulphides and fungicides derived therefrom. U.S. Rubber Co. (B.P. 816,341, 29.3.56. Can., 6.5.55).—A polymeric bisthiuram disulphide containing recurring units represented by ·S·CS·NH·R·NH·CS·S· (R is divalent ethylene, propylene, phenylene or the radical $[CH_2]_2$·N·$[CH_2]_2$·CS_2) is heated with alkali metal cyanide (0·5—1·5 mol.), to give a product of improved fungicidal activity. E.g., a 19% aq. solution of Na_2 ethylenebisdithiocarbamate is added simultaneously with an aq. solution of $(NH_4)_2S_2O_8$ to water, while keeping at 16—19° and at pH 6—6·6. When the pH drops to 4, the pptd. polyethylene thiuram disulphide is filtered off. This is treated with aq. NaCN at 25° and at <pH 8, to give material m.p. 157—170°, analysing S 51·3, N 17·5, C 27·3, H 3·12%. The substance is more active against tomato blight than is the original polymer. F. R. Basford.

Insecticidal compositions. Shell Research Ltd. (Inventor: P. Gerolt) (B.P. 815,511, 7.5.56).—Aldrin, dieldrin, endrin or isodrin is compounded (2—1) with a solid solvent (1—2 pt.) to provide an insecticidal composition which after application to a surface does not lose its activity. The composition may be further mixed with surface-active agents, and for use may be dispersed or dissolved in a water-insol. org. liquid and added to water if desired. The solid solvent, specifically of m.p. 50—250°, may be a hard, grindable, brittle substance (with no definite crystal lattice), and may be selected from S, resin (coumarone-indene resin or polychlorinated polyphenyl), asphaltic bitumen, petroleum or coal tar pitch, or an asphaltite (e.g., Gilsonite). F. R. Basford.

Thiophosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 816,286, 3.1.58. Ger., 5.1. and 16.12.57).—Compounds CN·CH;CR·CH_2·S·PX$(OR')_2$ and insecticidal compositions from them are claimed (R is H or alkyl of 1—4 C; X is O or S; R′ is alkyl of 1—4 C). The prep. is described of OO-*diethyl* S-3-*cyanoprop*-3-*enyl*

phosphorothionate b.p. 94°/0·01 mm., in 82% yield. Emulsions containing 0·001% of this ester are 100% lethal to aphids, while red spider mites and their eggs are completely destroyed by aq. suspensions containing 0·01% of the products.
F. R. BASFORD.

OO-Dialkylphosphoric and OO-dialkylthionophosphoric acid esters of substituted halogenobutenols. Badische Anilin- & Soda-Fabrik A.-G. (Inventors: H. Pohlemann, H. Stummeyer and H. Adolphi) (B.P. 815,965, 1.1.58).—Compounds useful as insecticides of pronounced systemic activity are obtained by interaction of OR(OR')·PX·Y with CH_2:CY·CH(OH)·CH_2·X'R'', preferably in presence of acid-binding agent (R and R' are alkyl of 1—4 C; X is O or S; Y is halogen; X' is O, S, SO_2, or SO; R'' is H or saturated or unsaturated alkyl of 1—4 C). The prep. is detailed of *Et*$_2$ *2-chloro-4-methoxybut-1-en-3-yl phosphorothionate*, b.p. 96°/0·001 mm.
F. R. BASFORD.

Thiophosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 816,005, 7.9.56. Ger., 7.9.55).—Compounds $(OR)_2$ PX·S·A·S·CH_2·YR', useful as systemic insecticides, are obtained by interaction of R'Y·CH_2·S·A·Z with a salt of $(OR)_2$·PX·SH in an inert solvent (R is alkyl or aryl; R' is alkyl or aryl; X and Y are O or S; A is alkylene of ≯4 C; Z is halogen). In an example, OO-*Et*$_2$ S-2-*(ethylthiomethylthio)ethyl phosphorothiolate*, b.p. 150°/0·05 mm., is prepared, which is 100% active against aphids and spider mites at 0·001% concn.
F. R. BASFORD.

New thiophosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 815,218, 13.12.57. Ger., 21.12.56).—Compounds OR(OR'). PS·O·CHMe Bu^t (R and R' are alkyl of 1—4 C) are prepared by condensing OH·CHMe Bu^t with OR(OR')·PX·Y at 0—100 (60)° in presence of acid-binding agent (pyridine), optionally in a solvent (X is O or S; Y is halogen), then (where X is O) heating the product with S. The compounds are useful as insecticides (especially against flies, mites and aphids), and compositions containing (0·00001—1 wt.-% of) them for such use are also claimed. In an example, the prep. is described of *diethyl 3,3-dimethylbut-2-yl phosphorothionate* b.p. 61°/0·01 mm. The product is more active against spider mites, than, e.g., the but-2-yl and pent-2-yl analogues.
F. R. BASFORD.

Chloromethylated OO-dialkyl thiophosphoric acid esters. Farbwerke Hoechst A.-G. (Inventors: V. Scherer, H. Hahn and G. Stähler) B.P. 817,360, 24.9.57).—The prep. is described of *O*-chloromethyl *OO*-diethyl thio- and dithio-phosphates for use as insecticides. The *thiothionophosphate* has b.p. 93—95°/1 mm. H. S. R.

Emulsifiable toxicant compositions. Witco Chemical Co. Inc. (Emulsol Chemical Corp.) (B.P. 815,001, 4.9.56. U.S., 7.9.55).—An emulsifying agent suitable for prep. of concentrates of toxicants (herbicides, pesticides) which are readily dispersible in water of varying degrees of hardness) comprises a non-ionic surface-active agent [polyoxyethylene derivative of an alkylphenol (alkyl of 6—25 C; 9—30 oxyethylene groups), a fatty acid of 10—22 C (12—30 oxyethylene groups), or an alkanol of 9—22 C (12—30 oxyethylene groups)] and an anionic surface-active component. Specifically, the latter is constituted by at least one salt of an aromatic (alkylbenzene) sulphonic of which ≮15 (e.g., 40) wt.-% is the polyamine salt (2—4 C between amino groups). A typical formulation comprises: Bu 2,4-dichlorophenoxyacetate (78), kerosene (17) and emulsifying agent (5%) consisting of propylene diamine salt (23) of dodecylbenzenesulphonic acid, Ca dodecylbenzenesulphonate (2·5), Na petroleum sulphonate (2), propanol (15), high-boiling aromatic solvent (6·9) and non-ionic surface-active agent (50·6%) comprising a mixture of Tergitol-type compound (89·5) and a 1 : 10 mol. nonylphenol–ethylene oxide adduct (10·5%).
F. R. BASFORD.

Fungicidal compositions. Armour & Co. (B.P. 815,827, 25.6.56. U.S., 24.6.55).—A composition suitable for combating fungus diseases of plants comprises a carrier (org. solvent, water or inert powder), an emulsifying agent, and a compound NH_2·C(SR):NH,HX (X is Cl or Br; R is aliphatic hydrocarbon radical of 8—18 C), e.g., *S*-octylisothiouronium chloride or *S*-coco-isothiouronium chloride or bromide.
F. R. BASFORD.

[A] **Trichlorobenzoic acid.** [B] **Herbicidal mixtures.** Heyden Newport Chemical Corp. (B.P. 817,173—4, 21.10.55. U.S., 21.10.54. [B] divided out of [A]).—[A] A mixture of trichlorobenzoic acids is prepared (50—75% of 2,3,6-isomer and 2,4,5-isomer) by converting a crude 2,3,6-trichlorobenzyl halide (obtained by chlorination of a chlorotoluene) into a trichlorobenzyl ether which is then oxidised, e.g., with HNO_3. The product is useful as a herbicide. [B] The mixture contains ≯75% of the 2,3,6-isomer.
F. R. BASFORD.

Herbicide composition containing 2,2-dichlorobutyric acid or salts thereof. Dow Chemical Co. (Inventor: B. Van B. Toornman) (B.P. 815,200, 24.6.57).—A herbicidal composition of intermediate persistence comprises 2,2-dichlorobutyric acid (or a salt thereof, e.g., the Na salt) in admixture with a petroleum distillate, aq. emulsion, surface-active dispersing agent, or finely divided solid. The concn. of the active ingredient should be ≮0·001 wt.-%.
F. R. BASFORD.

Novel acetoacetanilides and acaricides therefrom. Cilag Ltd. (B.P. 817,356, 15.3.56. Switz., 30.3.55).—Compounds useful as acaricides comprise acetoacetanilides, viz., $AcCH_2$·CO·NREt (R is Ph substituted at $C_{(2)}$ by Me and optionally at $C_{(3)}$ by Cl). They are made by treating NHREt with diketen or with an alkyl (1—4 C) acetoacetate. *Acetoacet-N-ethyl-o-toluidide* has b.p. 172°/12 mm.
F. R. BASFORD.

Controlling plant growth. N.V. de Bataafsche Petroleum Maats. (Inventors: G. C. Vegter, J. T. Hackman, P. ten Haken and G. E. Barnsley) (B.P. 815,570, 30.12.57).—A composition for use in the control of the growth of plants (especially weeds) comprises a liquid or solid carrier (optionally admixed with wetting agent) and bis-(2,2,2-trichloro-1-hydroxy-ethyl) sulphide. If desired, there may also be present chloral, chloral hydrate and an additional herbicide (a pre-emergence herbicide).
F. R. BASFORD.

Control of undesired vegetation. Dow Chemical Co. (B.P. 815,210, 1.8.57. U.S., 2.8.56).—The composition comprises inert finely divided solid, wetting, emulsifying or surface-active agent, fertiliser and (as active ingredient) ≮0·001 wt.-% of a cyclohexane derivative substituted in the 1-position by C:X and by OY (X is H or Me; Y is H, Ac or COEt) and optionally in the 2-, 3-, or 4-position by Me, e.g., 1-ethynyl*cyclo*hexanol. Inert solid carrier is specifically diatomaceous earth, pyrophyllite, gypsum, kaolinite or neutral or acidic volcanic ash.
F. R. BASFORD.

Quaternary pyridylisoquinoline derivatives. Imperial Chemical Industries Ltd. (Inventors: W. R. Boon and R. F. Homer) (B.P. 815,348, 25.2.57).—Compounds useful as herbicides comprise diquaternary salts made by interaction of 1-(pyrid-2'-yl)isoquinoline or its 3,4-dihydro derivative with an ethylene glycol di-ester, e.g., ethylene dibromide or di-*p*-toluene-sulphonate (in an inert solvent or diluent, e.g. high-boiling polar liquid). 3,4-*Benz*-5,6-*dihydro*-NN'-*ethylene*-2,2'-*dipyridinium dibromide* is a hygroscopic solid. As an example of use, this (13) is admixed with wetting agent (19·5) and water (6500 pt.), to provide an aq. spray which (100 gal. per acre) is 100% lethal to *Brassica oleracea* var. *acephala*, *Beta vulgaris*, *Trifolium repens*, etc.
F. R. BASFORD.

3,4-Dihydro-1,3-benzoxazines. Dow Chemical Co. (Inventor: R. H. Rigterink) (B.P. 814,272, 9.8.57).—Compounds useful as bactericides, fungicides and herbicides, comprise 3,4-dihydro-1,3-benzoxazines substituted in the 3-position by cyclohexyl, benzyl, monochlorophenyl or dichlorophenyl and in the benzene nucleus by 1—2 Cl. As an example of prep., a mixture of paraformaldehyde KOH and Me OH is heated until homogeneous then the solution is cooled and *m*-chloroaniline followed by *p*-chlorophenol is added and the whole heated for 1 hr. at 83—85° to give 6-*chloro*-3-(m-*chlorophenyl*)-3,4-*dihydrobenzoxazine*, m.p. 103—104·5°.
F. S. BASFORD.

Nematodicidal compositions. Diamond Alkali Co., Assee of H. Bluestone and W. J. Pyne (B.P. 818,709, 11.11.55. U.S., 15.11.54).—The active ingredient of a nematodicidal composition (solid, solution or emulsion) is a tetrahydrothiophen-1,1-dioxide substituted by 1—3(*n*) halogen and by 4—*n* OH groups. Compounds specifically claimed include: 3,4-*dichloro*-, 3-*chloro*-, 3,4,*x*-*trichloro*-, 3,4-*dibromo*-, and 3-*chloro*-4-*hydroxy*-**tetrahydrothiophen-1,1-dioxide.**
F. R. BASFORD.

Animal Husbandry

[A] **Criteria for expressing nutritional values of subtropical grasses.** [B] **Nutritional values for 17 subtropical grasses.** R. Milford (*Aust. J. agric. Res.*, 1960, **11**, 121—137, 138—148).—[A] The results obtained from feeding experiments with 17 different grasses on penned sheep in Queensland are discussed. Existing temperate feeding standards cannot be used for the nutritional evaluation of these results. The most suitable criteria are digestible crude protein, N balance, and dry matter intake and digestibility. Seasonal fluctuations in nutritional value occur in subtropical grasses, and frost is an important factor in the decline of nutritional value. (32 references.)

[B] The nutritional value of each type of grass is assessed on the above basis.
S. G. AYERST.

Effect of chlorine dioxide on lignin content and cellulose digestibility of forages. J. T. Sullivan and T. V. Hershberger (*Science*, 1959, **130**, 1252).—Chlorine dioxide was passed through air-dry, finely ground roughage (orchard grass, reed canary grass or wheat straw) for up to 24 hr. The sample acquired an acid odour that could be

removed by aeration. In all cases the treatment decreased the acid-insol. lignin and increased the digestibility of the cellulose in the material. T. G. MORRIS.

Effect of several variables on *in vitro* rumen fermentation. D. C. Church and R. G. Petersen (*J. Dairy Sci.*, 1960, **43**, 81—92).—In *in vitro* rumen fermentation tests digestibility of cellulose and dry matter of Alta fescue increased with decreasing concn. of substrate and increase in rumen liquor, but was little affected by varying mineral supply. Source of rumen liquor affected substrate digestion. Variation in pH affected digestibility of cellulose and the optimum pH varied with rumen liquor source. Digestibility of lucerne dry matter and cellulose decreased with decreasing particle size, whilst that of peavine hay was unaffected. A. H. CORNFIELD.

Roughage intake and efficiency of feed utilisation in dairy cattle. J. B. Stone (*Dissert. Abstr.*, 1960, **20**, 2997—2998).—Individual cows varied widely in intake of roughage, even after adjustment for milk production, body wt. and change of wt. Cows with high consumption of one source of roughage also showed high consumption of other roughages. The average efficiency of the cows, calculated from consumption of food per unit wt. of milk, also varied widely. Efficiency was not correlated with age, or body wt.; it was higher in the early stages of lactation than in the later. Nutrients in grass silage were more efficient than those in hay. M. D. ANDERSON.

Utilisation of irrigated pastures by dariy cows. II. Effect of stocking rate. M. Freer (*J. agric. Sci.*, 1960, **54**, 243—256).—At a stocking rate of 1·0 and 1·2 cows/acre in two seasons 57 and 59% more milk was produced than at rates of 1·7 and 2·0 cows/acre. The solids-not-fat content in both milk and butter fat in the first season were depressed at the higher stocking rate. The yield per acre was 38—39% higher, whilst individual intake per cow was 14—20% lower at the heavier stocking rate but the gain in body wt. was lower. At the higher stocking rate cows consumed the grass as it grew, whilst at the lighter rate only 80% was consumed. M. LONG.

Effect of feeding ground hay and heated grains alone and in various combinations to cows upon rumen organic acids and fat content of milk. W. L. Ensor (*Dissert. Abstr.*, 1960, **20**, 2993).—Compared with normal hay–grain diets, those consisting solely of flaked maize, barley or oats, decreased the acetic acid content of the rumen of cows, and increased the propionic acid, so that these acids were present in about equal proportions. Butyric acid contents were not affected. Linseed oil meal or cottonseed oil meal as sole diet had a less marked effect on acetic and propionic acid contents than had flaked grain. Decrease of acetic acid and increase of propionic acid was also observed on diets of lucerne hay + steam-heated maize, and lucerne pellets + ground maize, or steam-heated or flame-rolled maize. On the last-named diet, butyric acid was increased. Diets of heated or non-heated lucerne pellets gave the same proportions of volatile fatty acids as did those of long lucerne hay. All diets decreasing acetic acid and increasing propionic acid contents also decreased milk-fat production, in some cases by 60%. A diet of lucerne pellets + glucose did not affect rumen fatty acids or milk fat. M. D. ANDERSON.

Physical and chemical changes in bovine saliva and rumen liquid with different hay–grain rations. R. S. Emery, C. K. Smith, R. M. Grimes, C. F. Huffman and C. W. Duncan (*J. Dairy Sci.*, 1960, **43**, 76—80).—The viscosity of cow saliva was greater 3 hr. after feeding than before feeding and its pH increased with the proportion of hay in the feed. Saliva ash, total salivary N and hexosamines were greatest with the low-hay ration. The Na concn. in the rumen liquid increased with hay content of the diet and was higher for the cow which tended to bloat than for the one which did not. Hexosamine concn. of the rumen liquid was also greater for the affected cow, and tended to increase in both cows with decreasing proportion of hay in the feed. Rumen-liquid total N was highest on the lowest hay ration (20% in the feed), was higher after feeding than before, and was higher for the affected than for the normal cow. A. H. CORNFIELD.

Rearing calves on sour skim milk. W. A. Verbeek (*S. Afr. J. agric. Sci.*, 1959, **2**, 579—580).—Calves were fed with sour skim milk at room temp. for at least 2 months. Supplementary feed was provided from the second week. The calves readily accepted the sour milk; no digestive disturbances were observed, and satisfactory gains were made. I. DICKINSON.

Influence of different nitrogen sources with differential fat and diethylstilboestrol levels on feed utilisation and fattening performance of lambs. R. J. Raleigh (*Dissert. Abstr.*, 1960, **20**, 2996).—Feeding to wether lambs 1·67 mg. of stilboestrol per lb. of pelleted food did not affect wt. gain, feed consumption, pelt wt., carcass shrinkage, digestibility of N or N balance; market and carcass grades and dressing % were decreased; digestibilities of dry matter and energy were increased. Protein supplied as urea–beet molasses gave, in most cases, better wt. gains, market grade, pelt wt., dressing %, and N digestibility, than did ammoniated beet-pulp; urea–cane molasses and cottonseed meal were of intermediate value. Increase of dietary protein from 7 to 11% increased wt. gain, N balance, carcass grade, and digestibility of dry matter, N, and energy. Addition of 4% of fat to the diet (otherwise containing none) had no effect when considered alone, but entered into interactions with other factors. The lambs suffered from urinary calculi, ate wool, and were unthrifty. M. D. ANDERSON.

Toxicity of urea to sheep. J. B. Coombe, D. E. Tribe and J. W. C. Morrison (*Aust. J. agric. Res.*, 1960, **11**, 247—256).—In tests on the rumen contents of sheep fed diets with and without urea the rumen pH and ammonium levels were recorded. Rumination time declined as the pH level rose. Up to 100 g. of urea per day were taken readily, provided that the concn. in the ration was low. Much lower quantities given as a drench produced toxic signs. The influence of rumen pH level on the rate of ammonium absorption is discussed. (17 references.) S. G. AYERST.

The skeleton of the sheep. IV. The effects and interactions of dietary supplements of calcium, phosphorus, cod-liver oil and energy, as starch, on the skeleton of growing blackface wethers. D. Benzie, A. W. Boyne, A. C. Dalgarno, J. Duckworth, R. Hill and D. M. Walker (*J. agric. Sci.*, 1960, 54, 202—221).—Ca was the most important supplement in increasing the ash content of all bones. Cod-liver oil had a smaller effect and P still less. Positive interactions occurred with Ca/cod-liver oil and cod-liver oil/energy supplements, the former applying to the ribs and vertebrae and the latter to the long bones in particular. Starch was generally harmful, but did not affect the ash % in the mandible. P raised the ash of the lower vertebrae, ribs and some long bones, whilst cod-liver oil increased that of the cervical vertebrae and bones of the head. Ca increased long bone length as also did cod-liver oil/starch. M. LONG.

Comparison of meal and pelleted forms of creep feed for suckling pigs. R. Braude, M. J. Townsend and J. K. Rowell (*J. agric. Sci.*, 1960, **54**, 274—277).—No difference in food consumption or gain in wt., was attributable to the form of the diet but pigs preferred pellets to meal or to ground pellets. M. LONG.

Effect of adding lysine and methionine to the diet of pigs on low protein vegetable foods. R. E. Evans (*J. agric. Sci.*, 1960, **54**, 266—273).—Addition of 0·2% and 0·3% of lysine, together with 0·1% of methionine in both cases, in rations for pigs in the 36—80 lb. live wt. range reduced the mean meal requirements from 3·29 lb. to 2·97 and 2·86 lb. respectively per lb. live weight. This was maintained in the 80—120 lb. range when a lysine supplement of 0·64% reduced meal conversions from 3·34 to 3·07 lb. In N balance trials pigs receiving a lysine supplement retained 43·2% of food N compared with 34·5% by controls. A cereal diet supplemented with 7% fish meal produced a 44% retention of food N. M. LONG.

Techniques used in metabolism studies with surgically modified hens. C. E. Richardson, A. B. Watts, W. S. Wilkinson and J. M. Dixon (*Poultry Sci.*, 1960, **39**, 432—440).—Hens with exteriorised recta were preferable to hens with exteriorised ureters for metabolism studies. A method for the quant. collection of faeces and urine from such hens is described. Methylcellulose (1% in the diet) was a good binder for purified diets. H_3BO_3 was a suitable preservative for preventing loss of certain nitrogenous constituents during collection of urine and faeces. A. H. CORNFIELD.

Metabolic and anatomic adaptations in chickens trained to eat their daily food in two hours. S. Lepkovsky, A. Chari-Bitron, R. M. Lemmon, R. C. Ostwald and M. K. Dimick (*Poultry Sci.*, 1960, **39**, 385—389).—Chickens trained to eat their total daily food in 2 hr. adapted largely at the crop, enabling them to hold much more food, thus requiring little metabolic adaptation. Digestion proceeded more rapidly in untrained than in trained birds. During the height of digestion more proteolytic activity was drained from the pancreas and more glycogen accumulated in the livers of untrained than of trained birds. There were no differences in lipogenesis in various tissues between trained and untrained birds. A. H. CORNFIELD.

Apparent inhibitor in barley influencing efficiency of utilisation by chicks. G. H. Arscott, R. J. Rose and J. A. Harper (*Poultry Sci.*, 1960, **39**, 268—270).—Replacing the 61% of maize in a mash diet with barley considerably reduced wt. gains at 4 weeks, reduced feed efficiency, and considerably increased accumulated droppings. Chick performance on the barley diet was satisfactory when the barley was soaked and dried, autoclaved–soaked–dried, or soaked–dried–autoclaved before incorporation in the feed or when an

amylolytic enzyme was also added to the feed. Autoclaving the feed containing the enzyme destroyed the beneficial effect of the enzyme. A. H. CORNFIELD.

Food intake, water intake and body water regulation. S. Lepkovsky, A. Chari-Bitron, R. L. Lyman and M. K. Dimick (*Poultry Sci.*, 1960, **39**, 390—394).—Feed intake by chickens was not affected by withholding water. Crop water content was greater where water was supplied, but the water content and proteolytic activity of the intestinal contents were not affected by withholding water. Rate of digestion was slower when water was withheld. Withholding water decreased somewhat the accumulation of glycogen in the liver and leg muscles. A. H. CORNFIELD.

Thiamine requirement of growing chickens as influenced by breed differences. P. A. Thornton (*Poultry Sci.*, 1960, **39**, 440—444).—S.C. White Leghorns chicks required 0·0004 g. and New Hampshire × Delaware (heavy breed) chicks required 0·0002 g. of thiamine hydrochloride per lb. of feed to prevent mortality and promote max. growth. A. H. CORNFIELD.

Sulphur amino-acid requirement of the chick. T. S. Nelson, R. J. Young, R. B. Bradfield, J. B. Anderson, L. C. Norris, F. W. Hill and M. L. Scott (*Poultry Sci.*, 1960, **39**, 308—314).—The requirement of chicks to 6 weeks of age for S-amino-acids (methionine + cystine) was 3·51 ± 0·025% of the protein on diets containing 19·4—22·4% protein and 1380—1510 kg.-cal. of metabolisable energy per lb. A. H. CORNFIELD.

Thiamine requirement of young turkey poults. R. C. Robenalt (*Poultry Sci.*, 1960, **39**, 354—360).—Turkey poults required 100 μg. of thiamine per 100 g. of feed to prevent deficiency symptoms and ensure optimum growth to 12 days of age. Chicks had a very similar requirement. Poult growth between 12 and 22 days of age was better with 200 μg. than with 100 μg. of thiamine per 100 g. of feed, but this may have been due to loss of thiamine in the stored feed. A. H. CORNFIELD.

Effect of irradiated vegetable oils and animal fatty tissue and storage of the diet on growth and mortality in chicks. S. J. Ritchey and L. R. Richardson (*Poultry Sci.*, 1960, **39**, 404—408).—Irradiating soya-bean oil and maize oil with γ-rays did not affect their nutritive value when added to chick diets (10% level) either immediately after treatment or after 3 weeks' storage at room temp. Irradiation of beef fatty tissue prior to incorporation in the chick diet resulted in poor growth and high mortality with all periods of storage. Untreated and irradiated pork fatty tissue supported satisfactory chick growth when added fresh to the diet, but both materials caused increasingly poor growth and higher mortality with time of storage before addition to the diet. Addition of an antioxidant to the diets containing control and irradiated pork and irradiated beef fatty tissues resulted in normal performance of chicks. The poor performance of birds receiving pork fatty tissue was due to destruction of vitamins during storage of the diets. A. H. CORNFIELD.

Maize fermentation solubles in poultry nutrition. S. T. L. Tsang and P. J. Schaible (*Poultry Sci.*, 1960, **39**, 251—257).—Maize fermentation solubles (fermented maize extractives) supplied unidentified growth factor(s) required by chicks for rapid growth on practical broiler diets and also provided utilisable protein and energy. The material replaced an equiv. amount of menhaden fish meal in maize–soya-bean oil-meal rations with respect to unidentified growth factors. The greatest stimulating effect occurred with chicks on diets containing over 1000 kg.-cal. of productive energy per lb. of feed. A. H. CORNFIELD.

Emulsified oil as a liquid feed supplement for poultry. B. E. March and J. Biely (*Poultry Sci.*, 1960, **39**, 279—281).—An emulsion containing 10% of maize oil, given to 3—4-week-old chicks instead of drinking water, was well tolerated and was consumed in amounts to comprise 23·8% and 16·4% of the total feed intake when the basal diets contained 27·5 and 21·5% of protein respectively. Emulsions may be useful for supplying high levels of oil and eliminate the difficulties associated with the mixing and storing of oily diets. A. H. CORNFIELD.

Studies with laying hens. I. Effect of dietary fat, protein levels and other variables in practical rations. G. F. Combs and N. V. Helbacka (*Poultry Sci.*, 1960, **39**, 271—279).—Tests over 24—32 weeks with laying hens fed diets containing 14·6—19·1% protein and 909—1096 kg.-cal. productive energy per lb. and kg.-cal./protein (C/P) ratios ranging from 55 : 1 to 67 : 1,,are reported. The widest C/P ratio was adequate to support 60% egg production. Birds permitted free choice of feeds selected those with C/P ratios of 57—62 : 1. Addition of either hydrolysed animal and vegetable fat 9, animal tallow 10 or maize oil 10% significantly increased egg size, but had little effect on other factors. Presence of 0·6 g. of choline per lb. of feed was adequate even with diets containing 10% added fat. Addition of Zn (48 p.p.m.) to the diet had no effect except for a slight increase in egg yolk lipins. Addition of 1·5% of NH_4Cl did not affect egg production over 6 weeks but reduced egg shell thickness. A. H. CORNFIELD.

Radionuclide mineral studies: relation of breed, age and vitamin B_{12} to inorganic sulphur-35 metabolism in chicks. W. G. Martin and H. Patrick (*Poultry Sci.*, 1960, **39**, 282—286).—The sternum was generally the most active site of incorporation of SO_4^{2-} in bone tissue by chicks. White Leghorn chicks showed higher uptake rates of SO_4^{2-} than did New Hampshires or a cross between the two breeds. Addition of vitamin B_{12} to a purified soya-bean protein type ration increased uptake and retention of SO_4^{2-} by the chick whilst its addition to a practical ration reduced the chick's ability to incorporate SO_4^{2-}. A. H. CORNFIELD.

Restricted feeding of broiler-type replacement stock. R. E. Isaaks, B. L. Reid, R. E. Davies, J. H. Quisenberry and J. R. Couch (*Poultry Sci.*, 1960, **39**, 339—345).—Restricting feed intake from 8—9 to 21 weeks of age saved 25% of feed, whilst "restriction" by adding 15—20% fibre to the diet increased feed consumption by 39—49%. Restriction retarded growth during the rearing period, but had no effect on body wt. at 57 weeks of age. Feed restriction delayed sexual maturity, in proportion to severity of restriction, by 3 to 22 days. Feed restriction increased egg production in one test and had no effect in another. The % of hatchable eggs was increased in the former but unaffected in the latter test by feed restriction. A. H. CORNFIELD.

Use of lupin seed meal in chick rations. L. G. Swart and C. R. Liebenberg (*S. Afr. J. agric. Sci.*, 1959, **2**, 543—549).—The extent to which sweet lupin seed can be used in plant protein chick rations and the effect of supplementing plant protein rations, containing lupin seed meal, with DL-methionine (0·3%), vitamin B_{12} (5 μg. per lb.) and Aureomycin (5 mg. per lb.) were determined. Growth was stimulated by the addition of either methionine or vitamin B_{12}, but was not equal to that obtained with fish meal. Lupin seed meal had no detrimental effect on livability or feather growth but rations containing the meal as the main source of protein did not produce satisfactory growth. (16 references.)
I. DICKINSON.

Chick-growth inhibitor in lucerne. P. D. Clary, R. Gordon, D. Singman and S. Lepkovsky (*Poultry Sci.*, 1960, **39**, 399—404).—Some lucerne extracts showing high haemolytic activity did not depress growth of chicks, whilst other extracts showing weak haemolytic activity depressed growth. The growth-inhibitor in lucerne showed moderate lability to water so that it disappeared from lucerne extracts whilst they still contained considerable amounts of saponin (characterised by haemolytic activity). Lucerne extracts devoid of growth-depressing activity decreased blood-cholesterol of birds fed a diet without added cholesterol, but where cholesterol was added to the feed the treatment increased blood-cholesterol. A. H. CORNFIELD.

Effect of season and age of bird on egg size, quality and yield. F. E. Cunningham, O. J. Cotterill and E. M. Funk (*Poultry Sci.*, 1960, **39**, 289—299).—Characteristics of eggs laid over one year by hens, varying in age initially from 4 to 15 months, are reported. Egg wt. tended to be greatest during spring and lowest during periods of hot weather. The yolk : albumin ratio was not influenced by season but increased with age of bird. The decline in Haugh units was due entirely to ageing of birds and was not related to season. Total solids (%) in the albumin declined with time. Yield of albumin solids per egg tended to highest during March—April and was greater in eggs produced by older birds.
A. H. CORNFIELD.

Cholesterol content of eggs from various breeds and/or strains of chickens. H. M. Edwards, jun., J. C. Driggers, R. Dean and J. L. Carmon (*Poultry Sci.*, 1960, **39**, 487—489).—There were significant differences in % fat, % cholesterol and I value of the fat of eggs between eggs from 8 strains of chickens. There were no differences due to strain in % dry matter in the eggs. There were significant differences for hens within a strain in % dry matter and % cholesterol in the fat of eggs. A. H. CORNFIELD.

Lack of effect of egg yolk in the diet on the development of Rous sarcoma in the chick. M. S. Shorb, H. M. Walker and O. D. Keene (*Poultry Sci.*, 1960, **39**, 409—417).—Egg yolk diets and high-fat diets failed consistently to influence the time of appearance of Rous sarcoma tumours or time of death of chicks due to the tumours. In two tests, the rate of appearance of tumours and rate of death were increased, the common factor probably being the high fat content of the diet. A. H. CORNFIELD.

Effect of season and age of bird on the chemical composition of egg white. F. E. Cunningham, O. J. Cotterill and E. M. Funk (*Poultry Sci.*, 1960, **39**, 300—308).—The minerals and protein

contents of whites of eggs laid during 1 year by hens, varying in age initially from 4 months to 15 months, are reported. There were seasonal variations in Na, Ca and Cl but not in K, P and protein contents. P, Cl and protein contents varied particularly with age of bird, Ca content was affected slightly, whilst Na and K contents were unaffected. After the birds were a year old there was a good correlation between Haugh units and protein content of the albumin. None of the other factors was related to egg quality.
A. H. CORNFIELD.

Continuous feeding of hygromycin as a poultry anthelmintic and its effect upon laying. R. G. Foster, III, C. B. Ryan, R. D. Turk and J. H. Quisenberry (*Poultry Sci.*, 1960, **39,** 492—499).—Continuous feeding of hygromycin B (6—12 g. per ton of feed) from birth effectively reduced the no. of parasitised birds under conditions of relatively severe exposure. The 12-g. level produced the heaviest body wt. at 20 weeks of age, decreased mortality and reduced the no. of days to first egg. The treatments increased egg production. Feed efficiency with respect to egg production was highest with the 6-g. level. A. H. CORNFIELD.

Differences in susceptibility of Broad Breasted Bronze and Beltsville Small White turkeys to dietary-induced pendulous crop. H. O. Wheeler, B. L. Reid, T. M. Ferguson, J. R. Couch and R. H. Rigdon (*Poultry Sci.*, 1960, **39,** 263—267).—Broad Breasted Bronze turkeys on a glucose monohydrate-soya-bean protein diet exhibited 100% pendulous crop and 50% mortality at 10 weeks of age. Beltsville Small White turkeys on the same diet exhibited no pendulous crop but had 100% mortality at 10 weeks.
A. H. CORNFIELD.

Effect of continuous treatment with reserpine on body temperature, respiratory-cardiovascular functions, and heat tolerance of the hen. H. S. Weiss (*Poultry Sci.*, 1960, **39,** 366—373).—Addition of Serpasil (reserpine CIBA) (0·002 g. per kg. of feed) to the diet of White Leghorn hens for 32 weeks had no effect on body wt. or egg production. No overt tranquillisation was noted, but body temp., respiratory rate, heart rate and blood pressure were slightly depressed. Birds subjected to heat stress showed better survival time when they had been pre-treated with reserpine.
A. H. CORNFIELD.

Histological changes in the combs and ear lobes of chickens treated with methylcholanthrene. C. G. Crispens, jun., and H. L. Eastlick (*Poultry Sci.*, 1960, **39,** 448—454).—Histological changes in the combs and ear lobes of White Leghorn and White Silkie bantam chicks treated with methylcholanthrene (a carcinogen) are reported. A. H. CORNFIELD.

Blepharoconjunctivitis in turkeys. V. L. Sanger, E. N. Moore and N. A. Frank (*Poultry Sci.*, 1960, **39,** 482—487).—Symptoms of this enzootic disease of turkeys appear almost entirely during colder seasons. Mortality is low, but egg production and fertility may be reduced seriously. Streptomycin was effective in curing the trouble in limited trials. The condition disappeared when the weather turned warmer or when birds were placed in heated quarters.
A. H. CORNFIELD.

Pharmacologically-induced resistance to heat shock. I. Rauwolfoids and chlorpromazine. II. Modifications of activity of the central-nervous and endocrine systems. R. E. Burger and F. W. Lorenz (*Poultry Sci.*, 1960, **39,** 468—476, 477—482).—I. Reserpine, other rauwolfoids present in reserpine-free mother liquor, and chlorpromazine added to the diets were highly effective in delaying mortality of chickens subjected to experimental heat shock.

II. Sympatholytic agents and morphine added to the diets delayed the mortality of chickens subjected to experimental heat shock. When administered parenterally these drugs, and also general sedatives, either failed to protect or were damaging to survival. Glucocorticoids and also a short period of fasting afforded some protection. A. H. CORNFIELD.

Rapid assay of inorganic phosphates for chicks. C. B. Ammerman, H. W. Norton, H. M Scott and A. H. Nesbit (*Poultry Sci.*, 1960, **39,** 245—250).—The method described using day-old chicks and employing a 4-day P-depletion period followed by a 6-day P-supplementation period was satisfactory for assaying inorg. P supplements for chicks. A. H. CORNFIELD.

Distribution of the major nitrogenous compounds and amino-acids in chicken urine. B. L. O'Dell, W. D. Woods, O. A. Laerdal, A. M. Jeffay and J. E. Savage (*Poultry Sci.*, 1960, **39,** 426—432).—Uric acid accounted for 81% and NH_3 for 10% of the total N in the urine of 5—6-week-old White Leghorns. The proportion of urea increased when free arginine was added to the birds' diet, but creatine–creatinine-N and distribution of amino-acids was not affected by diet. Amino-acid-N accounted for about 2% of the total N. The amino-acids found in largest amounts were glycine, proline and glutamic acid. A. H. CORNFIELD.

Spectrophotometric method for the determination of uric acid in poultry excreta. G. S. Buys and D. J. J. Potgieter (*S. Afr. J. agric. Sci.*, 1959, **2,** 499—506).—Uric acid is extracted with 0·5% Li_2CO_3 solution at room temp. and centrifuged. The uric acid is pptd. as mercuric urate, redissolved in NaCl (0·5M in 1% acetic acid) and the absorbance is measured at 290 mμ. Recovery of uric acid from sheep faeces and poultry excreta exceeded 98%. The destruction of uric acid during hot extraction with Li_2CO_3 is severe.
I. DICKINSON.

Malathion dust-bath for control of five species of lice on chickens. J. L. Rodriguez and L. A. Riehl (*J. econ. Ent.*, 1960, **53,** 328).—A 4% malathion dust eradicated lice within 14 days and the chickens remained free for at least 84 days. C. M. HARDWICK.

Malathion dust control for chicken mite control. J. L. Rodriguez and L. A. Riehl (*J. econ. Ent.*, 1960, **53,** 328—329).—A 4% malathion dust applied by a hand duster, eradicated *Dermanyssus gallinae* from pens within 7 days and was residually effective for 5 months.
C. M. HARDWICK.

Colorimetric method for determination of Co-Ral in animal tissues. H. V. Claborn, M. C. Ivey and H. D. Mann (*J. econ. Ent.*, 1960, **53,** 263—265).—The method is based on heating Co-Ral in dilute Na_2CO_3 solution to open up the lactone ring of the umbelliferone radical and the reaction of the phenolic compound produced in an alkaline oxidising medium to give a red compound. A method for the separation of Co-Ral from animal tissues is also given. It is sensitive to 0·1 p.p.m. in a 50-g. sample. C. M. HARDWICK.

Residue studies of livestock sprays containing Sevin. R. H. Roberts, J. B. Jackson, W. E. Westlake, A. J. Ackerman and H. V. Claborn (*J. econ. Ent.*, 1960, **53,** 326—327).—No Sevin was found in milk between 5 hr. and 4 days after four spray applications. No residue of Sevin or 1-naphthol were found in the omental fat of beef cattle. C. M. HARDWICK.

***In vivo* activity of several systemic insecticides against cattle grubs in South Dakota.** W. M. Rogoff, P. H. Kohler and R. N. Duxbury (*J. econ. Ent.*, 1960, **53,** 183—187).—Compounds (11) were tested as sprays and by oral administration for control of *Hypoderma lineatum* and *H. bovis* over 5 years. Ronnel orally or as a drench was excellent except where infestation was very low. Dimethoate orally and intramuscularly was effective but depressed cholinesterase levels. Bayer 21/199 spray gave poor to excellent results only partially explainable by incomplete coverage. Dowco 109 spray was much less effective at 0·5% than 0·75% levels. There was no general wt. gain following grub control. C. M. HARDWICK.

Toxaphene residues in hogs. R. H. Roberts and R. D. Radeleff (*J. econ. Ent.*, 1960, **53,** 322).—No toxaphene was found in omental fat 4 or 6 weeks after one or two sprayings with 0·5% toxaphene. Toxaphene was found in renal fat after 4 weeks but not after 6 weeks. These residues were higher after two sprayings.
C. M. HARDWICK.

Livestock feeds. International Research & Development Corp. (Inventor: G. C. Cavanagh) (B.P. 817,834, 18.10.55).—An improved livestock feed which is convenient to handle in comminuted form and is readily compacted into pellets, cakes, etc., is economically produced by comminuting a vegetable source material, e.g., cotton seed, freeing from oil and fatty acids, then coating with ≯1 wt.-% of soapstock (derived, e.g., from the extracted fatty acids).
F. R. BASFORD.

Animal feed supplements. Biochemie G.m.b.H. (B.P. 818,675, 14.12.55. Aus., 14.12.54).—There is claimed an animal feed supplement comprising a penicillin degradation product (obtained by treatment of penicillin with alkali) and an accessory food factor or a basic feeding stuff. If desired, the composition may also contain an antibiotic. F. R. BASFORD.

Production of feed for ruminant animals. Sugar Research Foundation Inc. (Inventors: H. B. Hass, K. M. Herstein and M. Farber) (B.P. 815,317, 30.1.56).—A proteinaceous feed suitable for ruminant animals is prepared by treating pectin-containing solid agricultural material, especially finely-divided sugar-beet pulp, with NH_3 at −30° to +50°/500—800 mm. F. R. BASFORD.

Vitamin-E-fortified animal feeds. Eastman Kodak Co., Assee of J. G. Baxter (B.P. 815,414, 20.6.57. U.S., 28.6.56).—Vegetable oil (cottonseed and/or soya-bean oil) deodoriser sludge containing ≯0·2 wt.-% of α-tocopherol is acylated under anhyd. conditions (e.g., with Ac_2O), to acylate all of the tocopherol, then fatty acid present is converted into alkali metal- or alkaline-earth metal-soaps, and the resulting product is blended with inert solid (of 10—100 in. mesh), e.g., soya-bean meal, or hydrogenated fat, to provide a dry, free-flowing product useful as a vitamin-E supplement in animal feed. F. R. BASFORD.

Veterinary compositions containing cyanacethydrazide. Imperial Chemical Industries Ltd. (Inventors: N. Greenhalgh and J. K. Walley) (B.P. 818,467, 7.12.56).—A veterinary composition, for use in the treatment of lungworm infestations in domestic livestock, comprises an injectable aq. solution or suspension (of particle size $<100\mu$) containing cyanacetylhydrazide $\leqslant 0{\cdot}5$ (1—30)%, at least one other active ingredient (viz., an antibacterial, anthelmintic or antiprotozoal agent), and optionally a small amount of auxiliary agent (dispersing agent). F. R. BASFORD.

Veterinary compositions containing cyanacethydride. Imperial Chemical Industries Ltd. (Inventors: N. Greenhalgh and J. K. Walley) (B.P. 817,143, 817,358, 817,380, 7.12.56, [B] 21.9.56).—Compositions suitable for oral administration to domestic livestock suffering from lungworm infestations contain [A, C] 1—30%, [B] $\leqslant 0{\cdot}1$ (10%) of cyanacethydrazide [A] in an injectable vegetable oil (arachis oil), [B] mixed with a pulverulent diluent (talc, kaolin etc.) [C] in an aq. solution or suspension, with as stabiliser a S-oxy-acid or salt, e.g., $Na_2S_2O_5$. F. R. BASFORD.

Anthelmintic composition. Cooper, McDougall & Robertson Ltd. (Inventors: N. C. Brown, J. E. N. Slogan and P. A. Kingsbury) (B.P. 816,597, 20.12.57).—A veterinary or pharmaceutical anthelmintic prep. comprises a tasteless dichlorophen ester (preferably diacetate or dipropionate) and a suitable (edible) carrier. The prep. may be in the form of a pellet, pill or capsule, which, when incorporated into minced meat, is effective against *Taenia hydatigena* and *Dipylidium caninum* in dogs at a dose of 250 mg. per kg. F. R. BASFORD.

A stable phenylazo-diaminopyridine sulphonylthiourea complex. Farbenfabriken Bayer A.-G. (B.P. 817,531, 26.2.58. Ger., 12.3.57). —2,6-Diamino-5-phenylazopyridine is treated with 2 mol. of (*p*-aminobenzenesulphonyl)thiourea in a hot, water-sol. medium (MeOH, EtOH or dioxan), to give a 1 : 2 mol. *complex*, m.p. 140—142°, in the form of coarse, lustrous, orange-red crystals. The product is useful in the treatment of infections of the urinary tract in animals. F. R. BASFORD.

Veterinary compositions containing antibiotics. E. P. Beatson, J. C. Floyd and Imperial Chemical Industries Ltd. (B.P. 816,239, 13.8.56).—A non-aq. cream or paste suitable for use by intermammary injection in the treatment of mastitis, etc. comprises a suspension of penicillin and/or streptomycin and 2—3% of a dispersing agent (of hydrophilic-lipophilic balance 9·5—10·5, e.g., a sorbitan ester) in $\leqslant 30$ wt.-% of a suitable mineral oil, e.g., liquid paraffin or light liquid paraffin, or mineral oil thickened with white or yellow soft paraffin. Thus, a mixture of (93 pt.) liquid paraffin (58.5), white soft paraffin (38.5) and polyoxyethylene sorbitan monostearate (3%) is sterilised, then finely ground procaine penicillin of potency 1000 units per mg. is incorporated (7 pt.), to provide a veterinary composition. F. R. BASFORD.

[A] **Veterinary compositions containing organo-tin compounds.** [B] **Piperazinostannonium compounds.** Metal & Thermit Corp., Assee of L. E. Weinberg (B.P. 815,818—9, 24.12.57. U.S., 27.12.56). —Compounds $(SnR_mR'_n)X_{4-m}$ are claimed as anthelmintics for oral ingestion by fowls (m is 1—3; n is 1—2; R is a radical carrying (optionally) a non-toxic substituent, preferably with $\geqslant 22$ C; R′ is [A] an amino or heterocyclic basic group, [B] piperazino optionally substituted by an org. radical in the 2-, 3-, 5- and/or 6-position and in the 1- and/or 4-position by an innocuous radical; X is halogen, hydrocarbon-oxy or -mercapto, a carboxylic or mercapto-acid residue or an ester thereof, an arylsulphonic acid group, dithiocarbamate etc. group). They are prepared by interaction of, e.g., $SnBu_3$ acetate with the appropriate amine. Examples given are [A] di(phenothiazino)dibutylstannonium dilaurate and [B] *tributyl-piperazino)stannonium acetate*, m.p. 60—65°. F. R. BASFORD.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

The periodate-reactivity of the cellulose ethers (carboxymethylcellulose and oxyethyl-methyl cellulose). R. Schnabel (*Naturwissenschaften*, 1960, **47**, 180).—Aq. solutions (40%) of carboxymethylcellulose (**I**), oxyethyl-methyl cellulose (**II**), sucrose (**III**) and glucose (**IV**) have been reacted with 0·5% periodic acid solutions (unbuffered and buffered with Na acetate) at 1 : 1 vol. ratio, adding after 5 min. 5 parts (vol.) of freshly prepared Schiff reagent, determining the colour after 15 min. The intensity of color is in the order **IV** > **I** > **II** > **III**, with very little difference between **I** and **II**. Control tests in the absence of the periodic acid gave negative results. M. LAPIDOT.

Black-pointed wheat. Y. Pomeranz (*Bull. Res. Counc. Israel*, 1959, **7C**, 197—203).—Eight varieties of wheat grown in Israel showed different susceptibility to black point. Milling (low and medium extraction) of grist containing $\geqslant 13\%$ black-point kernels (**I**) does not affect flour colour, water absorption, dough-handling properties and bread quality. **I** show slightly decreased viability, but mould count and fat acidity did not differ much from those for sound wheat. W. J. BAKER.

Determination of moisture content in cereals. II. Errors in determination by oven drying of known changes in moisture content. T. A. Oxley and S. W. Pixton (*J. Sci. Fd Agric.*, 1960, **11**, 315—319; cf. J.S.F.A. Abstr., 1960, i, 189).—Water added to a soft wheat as vapour or liquid, was accurately assessed by the oven method (heating 4 hr. at 113°), but water added to a hard wheat was over-assessed proportionately to the amount added. When moisture content was increased from 9 to 25% (dry wt. basis), over-assessment was 1%. Water removed from both varieties by drying in a warm air current was over-assessed. Generally the ability of an oven-drying method to measure known quantities of water is related to the type of wheat, this finding being consistent with those of earlier work. E. M. J.

Examination of wheat gluten by partial solubility methods. I. Partition by organic solvent. II. Partition by dilute formic acid. P. Meredith, H. G. Sammons and A. C. Fraser (*J. Sci. Fd Agric.*, 1960, **11**, 320—328, 329—337).—I. Gluten from defatted flour was purified by dispersion in acid and pptn., and partitioned in a methanol–chloroform mixture. Similar partition of washed gluten and of flour gave results suggesting that part or all of the gluten-protein exists as a complex which is irreversibly split by acid treatment. The physical properties of the two fractions A and B were similar to those of gliadin and glutenin. The content of basic amino-acids is low in fraction A and its low ionic character accounts for the solubility in the methanol–chloroform mixture. Comparison with data for fractions of the same type from barley show that fraction A and hordein have similar amino-acid composition but fraction B and hordenin differ. These differences in the amino-acid contents of the less-sol. fractions may indicate the structural components responsible for the characteristic properties of wheat gluten. (38 references.)

II. When gluten is treated with dil. acid (formic), swelling of the gelatinous component occurs with diffusion from the gel of the more sol. proteins. Three fractions obtained were studied by electrophoretic and η measurements and the findings are discussed in relation to the structure of gluten. From consideration of wheat and barley proteins, the unique properties of wheat dough depend on the structure of the gel protein and its content of glutamine, proline, lysine and alanine. (23 references.) E. M. J.

Course of enzymic degradation of wheaten flour proteins. M. Rohrlich and W. B. Th. Schulz (*Naturwissenschaften*, 1960, **47**, 181).—Column chromatography was carried out on Dowex 50 × 2 on a series of wheat protein fractions obtained by enzymic (peptic and tryptic) degradation. The fractions were native wheat protein in the flour, flour protein after three extractions with 70% ethanol (glutenin), and gliadin. No characteristic differences are observed in the chromatograms of flour protein and glutenin. The glutenin contained more neutral and basic peptides than the gliadin. Hydrolysis of some fractions and measurement of the ninhydrin colour intensity revealed peptides of statistically 2—6 amino-acid remnants. The proline content has been established as 3—10%. M. LAPIDOT.

Effect of atmospheric sulphur dioxide on wheat flour. W. R. Morrison and J. Hawthorn (*Chem. & Ind.*, 1960, 529—530).—Atmospheric SO_2 in city air can be absorbed by static flour, harming the physical properties of dough. Probably, the SO_2 effect would hardly affect the bulk of the flour in flour milling. O. M. WHITTON.

Acceleration of wheat flour ripening by infra-red radiation. L. Auermann (*Getreide u. Mehl*, 1960, **10**, 37—38).—Freshly-milled flours (72% extraction) from normal and germinated wheat were exposed on both sides of a 0·5 cm. layer to i.r. lamps of 500 W at distances of 20 and 30 cm. for 6- and 8-min. periods, the layer being moved gradually to and fro during exposure. Flour and bread quality were then compared with those of the untreated flour freshly milled and after ripening periods at 18—22° of 1 and 2 months. An i.r. exposure for 6 min. of the flour at its natural moisture content could satisfactorily replace a ripening period of 1 to 2 months, the treated flour being in some of its baking qualities superior to the naturally ripened flour. C. L. HINTON.

Applicability and efficiency of Al_2O_3 chromatography in examination of potato starch. II. M. Ulmann (*Ernährungsforschung*, 1960, **5**, 170—183; cf. J.S.F.A. Abstr., 1956, ii, 143).—A review of the author's published work in which the technique has been applied

to the investigation of fresh and old starch solutions, the effect of cotton wool and of cation-exchangers on the composition of dissolved starch, and the clarification of the nature of the membrane observed on swollen starch grains. (15 references.) P. S. ARUP.

Production of amylase from submerged mould cultures, and its use in the pectin industry. F. Baum (*Ernährungsforschung*, 1960, **5**, 159—163).—Amylase is much more efficiently produced by submerged than by surface cultures of *Aspergillus oryzae*. The filtered culture solution contains 98—99% of the amylase produced, and can be used directly (or after concn. by evaporation at 40°) for the removal of starch from apple-pectin solutions. The amylolytic activity of the prep. remains practically constant during several weeks at room temp. and pH 5—8. The prep. are inactive in pectin solutions at pH <2·5; they must be tested to ensure that no pectinase is present. P. S. ARUP.

Rôle of thiol and disulphide groups in determining rheological properties of dough made from wheaten flour. R. Frater, F. J. R. Hird, H. J. Moss and J. R. Yates (*Nature, Lond.*, 1960, **186**, 451—454).—Chemical analyses of flour and flour extracts suggest that, at any given protein content, the strength and elasticity of dough are related directly to the no. of intermol. –S·S– bonds (**I**) and to their rate of interchange with –SH groups. Doughs from wheats of different quality correspondingly differ in respect of either or both of these properties, both water-sol. proteins and gluten fractions of the flour being involved. Observed reactions of –SH groups with IO_3^-, Br O_2^-, ClO_3^-, $S_2O_8^{2-}$ and *N*-ethylmaleimide (**II**), and the action of IO_3^-, **II** and cysteine on dough rheology, indicate that improvers strengthen the dough by inhibition of –S·S– exchange-reactions and (with oxidising agents) through formation of new **I**. Oxidation of thiol groups is slower with BrO_3^- than with IO_3^-, so that a more stable structure develops during dough prep. and baking. Reducing agents, e.g., cysteine, destroy dough structure by decreasing the no. of **I** and/or increasing the rate of their exchange. W. J. BAKER.

Dough management with a primary- but without a full-sour. H. Huber (*Brot u. Gebäck*, 1960, **14**, 61—69).—Establishment of the scientific basis of the single-stage leavening of rye dough is described. Conditions for the production of the primary-sour were investigated. A min. % of starter is necessary; temp. may vary within wide limits, the optimum being 24°, but it should not exceed 30°. A solid consistency is favourable to stability, as the development of undesirable organisms is suppressed. The primary-sour can retain its biological activity even for some days without being refreshed. The acetic acid content forms about 25% of the total acid content, comprising lactic and acetic acids, traces of succinic, malic, citric and formic acids. From baking tests it is shown that with the primary-sour process the flavour of the bread can be suitably adjusted by raising or lowering the proportion of sour; a normal proportion is 10—15% of the flour. Improved characteristics of rye and mixed breads prepared by the process are described. (25 references.) C. L. HINTON.

The development of the knife-slash in rolls from firm and soft doughs. J. Hoepfner (*Brot u. Gebäck*, 1960, **14**, 70—77).—Baking tests with doughs of varying consistency showed that the slashed roll requires a rather firmer dough than the plain roll. Over a narrow range, however, the consistency requirements overlap, and good-quality rolls of both types can thus be made from the same dough. C. L. HINTON.

Comparative baking tests with potassium bromate and Stimol, an improver prepared from whey. D. De Ruiter and W. H. G. Wiebols (*Bakkerswereld*, 1959/60, **20**, Repr. 6 pp.).—Stimol (like lard) is somewhat superior to $KBrO_3$ in the improvement of crumb-texture and -colour, but inferior as regards the improvement of dough-expansion and other respects. P. S. ARUP.

Formation, constitution and determination of elements of aroma in bread. M. Rothe and B. Thomas (*Nahrung*, 1959, **3**, 1—17).—The compounds which contribute mainly to the aroma of bread are aldehydes, particularly methyl aldehydes and amino-acid aldehydes. Their formation during rising and their reactions during baking are considered. A method for the determination of aldehydes in bread has been developed and results obtained for a series of different types of bread are tabulated and discussed. The taste values were found to be related to the aldehyde content and with increased baking time aldehyde content increases. Diffusion of volatile aldehydes from crust to crumb during storage has been observed and on longer storage parallel destruction of crumb aldehydes occurs. S. M. MARSH.

Volatile aroma-producing substances of rye bread. M. Rothe (*Ernährungsforschung*, 1960, **5**, 131—142).—The amino-acids chiefly involved in the production of aroma-forming aldehydes during baking, are alanine, valine, leucine, isoleucine, methionine and phenylalanine. When heated (under baking conditions) with xylose, these acids are converted (by a type of Maillard reaction) into the corresponding aldehydes which, in addition to the furfuraldehyde also formed, can be identified by chromatographic analysis of their respective dinitrophenylhydrazones. These findings are confirmed by chromatographic analyses of the volatile matter formed in comparative baking tests with dough containing added xylose or one of the above amino-acids. Methylglyoxal is formed by the decomposition of threonine. (12 references.) P. S. ARUP.

Avoidance of shrinkage cracks in macaroni drying. P. Görling (*Getreide u. Mehl*, 1960, **10**, 39—43).—Shrinkage cracks in macaroni during drying are formed as a result of tensions set up by shrinkage differences when the outer layers, with diminishing moisture content, lose their plastic properties and enter the elastic zone. Cracking can be avoided, and the process of drying speeded up, by a "sweating" procedure, viz., by drying at a high temp. when the dough is still largely in the plastic field and, at a point where the outer layers enter the elastic zone, raising the atm. humidity, considerably for a time, with lowering of temp., to allow equilibration to occur. The humidity is then gradually lowered until drying is complete. In fixing the conditions necessary, as required by the moisture equilibrium curve of the dough and the deformation characteristics in relation to moisture content, a method of measuring shrinkage tension in the surface layer was useful. By following the principles developed, residual tensions and liability to cracking are considerably diminished. Resistance of the goods to humidity and temp. changes during storage is also improved, especially when they are suitably packed. C. L. HINTON.

Bulk handling and automation at biscuit works. Anon. (*Engineer*, 1960, **209**, 1060—1062).—The bulk handling of flour and other ingredients at the works of Carr and Co., Ltd., is illustrated and described. C. V.

Derivatives of polysaccharides, more especially of starch, by a dry process. W. A. Scholten's Chemische Fabrieken N.V. (B.P. 815,358, 25.6.57. Neth., 26.6.56).—A dry process for the production of derivatives of polysaccharides, especially starch (potato, maize, cassava, wheat or sago starch, waxy starch or modified starch) or water-sol. gum, comprises treating the polysaccharide in dense fluidised phase with an etherifying, esterifying or acetalising agent in presence of a catalyst. Apparatus is figured. F. R. BASFORD.

Sugars and confectionery

Analysis of diluted saturation sludge in sugar production. I. Vavra and A. Vavra (*Kem. u Industr., Zagreb*, 1960, **9**, 33—42).—The practice of taking sludge samples directly past the press filters gave considerable variations in the results owing to the inhomogeneity of the samples. Sampling of the mixed bulked sludge prior to final disposal is recommended. The sample is diluted to sp. gr. 1·01—1·13 and analysed polarimetrically after treatment with aq. $ZnCl_2$. L. GROCHOWSKI.

Decolorising ion exchangers in sugar industry. J. Stamberg, V. Valter and Z. Mencl (*Chem. Tech., Berlin*, 1960, **12**, 32—36).—Diluted molasses solution was used in laboratory experiments, with resin in the chloride form. Decolorisation is strongly diffusion-dependent and best results are obtained at high temp. Optimum decolorisation conditions are given by the point of intersection of curves of swelling vs. amphoterity for acid, alkali and neutral regeneration. Laboratory, pilot-plant and full-scale technical apparatus were constructed. Two pilot plants were constructed, one for slightly and the other for strongly alkaline sugar solutions. Decolorisation of strongly alkaline solutions is best carried out using strongly basic resins and HCl activation. The first full-scale plant installed has a total resin vol. of 2 cu.m. Decolorisation is carried out at 80° with a sp. loading of ~1. Initial decolorisation is better than that obtained on a smaller scale but efficiency of the resin gradually decreases. Decolorisation using ion-exchangers is cheaper and simpler than other methods in use. M. G. SEAMAN.

Behaviour of aldehydes on a sulphonated polystyrene resin. S. A. Barker, K. Murray and M. Stacey (*Nature, Lond.*, 1960, **186**, 469—470).—Salicylaldehyde, 2-furaldehyde and 5-hydroxymethyl-2-furaldehyde are strongly retained on a column of Zeokarb 225 (H form). The effect can be utilised in the identification of intermediates in the browning reaction or of products of acidic degradation of sugars. W. J. BAKER.

Invertase and cast cream centres. F. Jansen (*Mfg Confect.*, 1960, **40**, No. 4, 41—42, 44, 56—57).—Refined commercial invertase prepared from yeast is flavourless and will keep indefinitely. The mechanism of inversion in the cream centres is discussed, the end point being adjusted to the type of centre required. Optimum

conditions in terms of pH, cooking time, sugar and syrup content, casting temp. and water content are indicated and formulae are given. C. V.

Separation of glutamic acid from [A] the residual liquid obtained after the production of sugar from sugar-containing juices [B] vinasse. N.V. Centrale Suiker Maats. (B.P. 817,178—9, [A] 13.8.56, [B] 4.2.57. Neth., [A] 20.8.55, [B] 18.2.56).—[A] Liquor from the production of sugar-containing juices, containing pyrrolidonecarboxylic acid (**I**), is (after the removal of most of the sugar) heated with alkaline earth metal oxide or hydroxide during 10—15 min. at 80—110°, then cooled and diluted with 1—3 pt. of an org. polar liquid (MeOH), optionally in aq. form. Pptd. solid is filtered off, then the filtrate is freed from org. liquid, and the aq. residue is concentrated to sp. gr. 1·2—1·4 ; **I** is hydrolysed (under acid or alkaline conditions) to form glutamic acid which is recovered in good yield (e.g., 72·5%) and in high (99%) purity by concentrating the hydrolysis liquor and adjusting the concentrate to the isoelectric point.

[B] Vinasse, diluted if necessary, is heated with alkaline-earth metal oxide or hydroxide at 50—100° without hydrolysis of **I** present, the mass filtered and the liquor heated with acid or alkali as in [A]. F. R. BASFORD.

Recovery of sugar from sugar-containing substances. F. Uhde G.m.b.H. (B.P. 818,341, 15.8.55. Ger., 28.4.55).—An improved process for the recovery of sugar from sugar-containing substances, especially dried sugar-beet chips, sugar-containing concentrates (molasses), or residues from the present process, comprises extracting with liquid NH_3, then treating the extract with an org. solvent (monohydric or dihydric alcohol of $\geq$5 C ; ketone of $\geq$5 C ; a formamide) optionally in presence of a small amount of water, selectively to precipitate high-mol. products. Sugar (or an NH_3 complex thereof) is recovered from the filtered liquor. F. R. BASFORD.

Fermentation and Alcoholic Beverages

Glucose–fructose ratio of table wines and its dependence on various cellar treatments. J. Koch and G. Bretthauer (*Z. Lebensmitt-Untersuch.*, 1960, **112**, 97—105).—For determination of glucose and fructose respectively in wines with <15 g./l. of total sugar the paper chromatographic method of E. Bayer (*Vitis*, 1958, **1**, 298) and the Cu reduction method (at 50°) of F. Prillinger (*Mitt. Wein u. Obstbau, Wien*, 1952, **2**, 20) were found suitable. Both methods are described. For series analyses it is preferable to determine fructose by Prillinger's method and total sugars by Luff–Schoorl ; glucose is then given by difference, less 1 g./l. correction for arabinose. In the prep. of table wines glucose is fermented more rapidly than fructose. A high glucose/fructose ratio (>0·66) cannot be used satisfactorily to detect after-sweetening with sucrose or grape juice except when added to almost fully fermented wine. (19 references.) C. L. HINTON.

Malolactic fermentation in wines and ciders. II. Growth promoting effect of yeast extract on lactic acid bacteria causing malolactic fermentation in wines. H. Lüthi and U. Vetsch (*J. appl. Bact.*, 1960, **22**, 384—391).—Yeast extract (**I**) and proteose promote growth of the species of *Lactobacillus* and *Leuconostoc* responsible for malolactic fermentation in wines. Treatment of **I** with 96% ethanol isolated the growth promoting components in the alcohol-sol. fraction. Ion-exchange experimentation indicated that the active substances (**II**) were probably simple N-compounds, possibly strepogenin-like in nature. With acid hydrolysis the activity is lost. **II** can be concentrated by paper chromatography. (10 references.) C. V.

Organic acid metabolism in cider and perry fermentations. II. Non-volatile organic acids of cider-apple juices and sulphited ciders. G. C. Whiting and R. A. Coggins (*J. Sci. Fd Agric.*, 1960, **11**, 337—344 ; cf. J.S.F.A. Abstr., 1956, i, 227).—The separation of these substances by ion-exchange resin absorption, silica gel- and paper chromatography and identification are described. Additional acids to those previously observed were mucic, benzoic, gluconic and 2-methyl-2,3-dihydroxybutyric. No acid decreased but malic acid sometimes increased in amount during sulphited fermentations. Gluconic acid may have been derived from a proportion of the sugar and the presence of comparatively large amounts of mono-, di- and tri-galacturonic acids indicated degradation of pectin. (35 references. E. M. J.

Special characteristics of cider lactobacilli. J. G. Carr (*J. appl. Bact.*, 1960, **22**, 377—383).—Comparison with other lactobacilli shows that the low pH of cider has imposed special characteristics on their energy metabolism. Sugar fermentation tests in acid media are thus more informative than those in neutral ones. Competition with yeasts is discussed and it is noted that even the polysaccharide produced by the only slime-forming rod isolated differs from the dextran which is usually encountered. (14 references.) C. V.

Carbon dioxide and oxygen tensions in steep waters. A. R. Chambers and A. D. B. Lambie (*J. Inst. Brew.*, 1960, **66**, [New Ser. **57**], 159—162).—Replacement of CO_2 in barley steep waters by O_2 tends to lead to bolting of the germinating seed, but satisfactory results were obtained with steeps containing 1400—1800 p.p.m. of CO_2 The presence of a certain concn. of CO_2 is useful in inhibiting premature growth and obtaining subsequent even germination. C. A. SLATER.

Shortening of dormancy of barley with gibberellic acid. W. Kleber, M. Lindemann and P. Schmid (*Brauwelt*, 1959, **99 B**, 1745—1746).—Preliminary work is reported in which the addition of 60 μg. of gibberellic acid to 500 freshly harvested barley grains (four varieties were tried) in the first steeping water released the full germinative energy (the H_2O_2 method being used as control). C. L. HINTON.

Small-scale malting experiments with gibberellic acid. W. Kleber, M. Lindemann and P. Schmid (*Brauwelt*, 1959, **99 B**, 1781—1785).—In preliminary experiments on 1-kg. lots of a single barley variety (Firlbeck III) an addition of 0·15 mg. gibberellic acid in the steep water was found to shorten the germination time by 2 days while the quality characteristics of the malt were at least equal to those of the normally malted sample. By spraying a solution of gibberellic acid on to the grain at the beginning of the germination period a quantity of 0·06 mg. sufficed to give the same saving in germination time, with improved malt quality in some respects. 11 references.) C. L. HINTON.

Kaffircorn malting and brewing studies. IV. Extraction and nature of the insoluble amylases of kaffircorn malts. V. Occurrence of β-amylase in kaffircorn malts. L. Novellie. **VI. Starch content of kaffir beer brewing materials. VII. Changes in carbohydrates of kaffircorn during malting.** M. M. von Holdt and J. C. Brand (*J. Sci. Fd Agric.*, 1960, **11**, 408—421, 457—463, 463—467, 467—471 ; cf. J.S.F.A. Abstr., 1959, ii, 266).—IV. The insol. amylases occurring in malts from " birdproof " kaffircorn and sweet sorghum are active in the insol. state. Their behaviour is compared with those of barley malts. Proteins, peptone, ethylenediamine, histidine and compounds containing the group –N·C(X)·N– (where X is not O) effect max. liberation of the insol. amylases. Partial liberation is obtained with salts, surface-active agents and basic substances (e.g., basic amino-acids, etc.). The insol. amylases seem to be adsorbed on the surface of a globulin or a nucleoprotein, their active centres being free to dextrinise and saccharify the starch. (28 references.)

V. South African sorghum malts in contrast with those of U.S. contain significant amounts of β-amylase which is formed during germination at the same rate as α-amylase, 18—30% of the saccharifying activity being due to β-amylase. In a purified state its properties are like those of other cereal β-amylases. The low diastatic activity of kaffircorn malts compared with those of barley and wheat is attributed to the lower proportion of β-amylase in the sorghum malts. (20 references.)

VI. As determined by the modified method of MacWilliam *et al.* the starch content of kaffircorn grains was 61·1—69% ; kaffircorn malts 45·9—61·2% and maize grits and maize meals 71·8 to 81·7%. The starch content of spent grains from different breweries varied from 14·7 to 50·3%. During a mashing trial 40·7% of the starch was not broken down ; of this, 7·9% was discarded in spent grains and 32·8% appeared in the finished beer, the η of which is an important factor in consumer acceptance. (13 references.)

VII. Sugars found in the kaffircorn grain were : glucose, fructose and sucrose ; in the malt (prepared by steeping grain for 8 hr. at 20—25°, allowing to germinate at 30° for 7 days and drying in forced draught for 24 hr. at 50°), fructose, glucose, sucrose and maltose. (12 references.) E. M. J.

Carbohydrates in malting and brewing. IX. Rapid method for estimating main carbohydrate constituents of worts. G. Harris and I. C. MacWilliam (*J. Inst. Brew.*, 1960, **66** [New Ser. **57**], 147—150).—Sucrose, maltose, maltotriose and dextrins in wort are estimated by specific enzymic hydrolysis followed by measurement of the amount of hexose monosaccharide formed by Barfoed's reagent. The method is considerably quicker than that which depends on chromatographic separation followed by the use of anthrone–H_2SO_4. (20 references.) C. A. SLATER.

[A] Observation on beer spoilage lactobacilli. [B] Improved membrane filter technique for rapid identification of spoilage organisms. J. O. Harris and W. Watson (*J. Inst. Brew.*, 1960, **66** [New Ser. **57**], 151—154, 154—159).—[A] A medium suitable for the quant. determination of lactic bacteria is described. Two strains of lacto-

bacilli capable of producing diacetyl in beer were isolated. (11 references.)

[B] A method for the identification of organisms isolated on membrane filters is described. This rapid and sensitive technique may be applied to the determination of low degrees of bacterial infection of yeast. C. A. SLATER.

Lactobacilli causing ropiness in beer. D. H. Williamson (*J. appl. Bact.*, 1960, **22**, 392—402).—Three strains resembling *L. brevis* were isolated; they differed in possessing a capsule and in their inability to produce ropiness in liquid culture. A new technique for estimating ropiness is employed, the ability to attain this state in a semi-defined medium depending on the optimum concn. of acetate together with an excess of a suitable source of C. Extracellular nucleic acid is found only in some ropy cultures but a polysaccharide (**I**) composed mainly of glucose is present in all. The degree of ropiness does not depend on the amount of **I** produced. (23 references.) C. V.

Rapid chromatographic-polarimetric determination of α-bitter acids (humulones) content of hops. H. Bausch, H. Wolter and D. Sommer (*Nahrung*, 1959, **3**, 501—514).—The chromatographic-polarimetric method of Verzele has been evaluated and shown to give low results in some cases compared with the Wöllmer and Gough methods. A modification of the Verzele method is proposed in which ethyl acetate through which SO_2 has been bubbled is used as eluate on a column of active C. Standard error is ±0·19%. The method requires only 30 min. to complete a determination. S. M. MARSH.

Determination of coagulable nitrogen in wort and beer. G. Franke (*Brauwelt*, 1959, **99 B**, 1637—1639).—An electrically controlled glycerin bath was designed to replace the customary salt bath in the determination of coagulable N by the method of Kolbach and Wilharm (*Wschr. Brau.*, 1934, **51**, 57). C. L. HINTON.

Cold-stabilisation of beer with nylon powder. C. Weymar (*Mschr. Brauerei wissen. Beil.*, 1960, **13**, 41—42).—Nylon powder, although unsatisfactory as a filter aid for unfiltered beer, when shaken with bottled beers of the Pilsener and Export types gave a marked improvement in clarity, colour and resistance to low temperature turbidity, and much diminished tannin content, while foam-holding ability and nitrogenous constituents of the beer remained unaffected. In technical practice the nylon powder could probably be most suitably applied at the kieselguhr filtration stage. C. L. HINTON.

Beer malt. Kurth Malting Co. (Inventor: E. Kneen) (B.P. 815,377, 30.1.56).—Gushing of beer is minimised by adding thereto 5—250 p.p.m. of EDTA or a salt thereof (e.g., the mono-Ca salt or a di-, tri- or tetra-Na salt). If desired, the EDTA may be incorporated in the green malt used in the production of the beer. The final product is also characterised by increased stability to oxidation. F. R. BASFORD.

Improvements in beer making. E. Barrey (B.P. 816,251, 8.1.57. Fr., 27.1.56).—To obtain a desired type of beer, the usual brewing operations are performed so as to produce a brewery liquid in which the values of some factors (especially alcoholic strength, % of solids and bitterness) are appreciably greater than those of the desired type. The brewery liquid thus obtained is stocked, then converted into the desired beer by adding (preferably at the time of consumption) water charged with CO_2/>1 atm. F. R. BASFORD.

Improved beer. A. Hansen and E. Helm, trading as A. Jorgensen's Gaeringsfysiologiske Laboratorium (B.P. 816,361, 1.5.57. Den., 16.5.56).—Tendency to gushing in beer is reduced by incorporating therein (in any product used in a manufacturing stage after the main fermentation) a non-toxic amount of a cobaltous salt of an org. or inorg. acid (to give >0·1 mg., e.g., 1·0 mg., of Co per litre), e.g., $Co(NO_3)_2$ or $CoCl_2$. F. R. BASFORD.

Fruits, Vegetables, etc.

Use of gas chromatography in measuring the ethylene production of stored apples. D. F. Meigh (*J. Sci. Fd Agric.*, 1960, **11**, 381—385).—A method is described using a sensitive flame ionisation detector and a column long enough to separate ethylene from possible interfering substances. In 0·5 ml. samples of air ethylene (<1 p.p.m.) was determined. The method was applied to measurements of ethylene from apples stored under various conditions. (12 references.) E. M. J.

Determination of 1-naphthyl methylcarbamate (Sevin) residues in apples. H. J. Hardon, H. Brunink and E. W. van der Pol (*Analyst*, 1960, **85**, 187—189).—The sample is extracted with methylene chloride and the residue from the evaporated extract is heated with acetonitrile. The diluted mixture is extracted successively with light petroleum and methylene chloride. The residue from the second evaporated extract is diazotised and coupled with sulphanilamide; extinction is measured at 520 mμ and referred to a calibration graph. Recovery of known amounts was 96%. A. O. JONES.

Storage loss of sulphur dioxide in ripe mango pulps preserved with bisulphites. A. N. Bose and S. B. Lodh (*J. Inst. Chem. India*, 1960, **32**, 9—13).—The rate of loss of available SO_2 in pulp from three varieties of mango stored at different temp. and pH and the effect on spoilage was determined. Rate of loss was less from a pulp preserved with 1000 p.p.m. than from one with lower concn. of SO_2. J. V. RUSSO.

Pickling of green olives. XVII. Yeasts present in fermentation. F. González Cancho (*Grasas y Aceites*, 1960, **11**, 9—18).—Seventy-one strains of yeast have been isolated from 43 different brines, corresponding to the genera *Candida, Hansenula, Pichia, Torulopsis* and *Saccharomyces*. Fermentative yeasts, found from the early stages of fermentation, were more common than oxidative types, which were found only in brines one or two years old. L. A. O'NEILL.

Proteins in olive seeds. M. J. Fernández Diez (*Grasas y Aceites*, 1960, **11**, 19—25).—The best conditions, particularly pH, for the extraction and pptn. of proteins from three varieties of olive seed have been studied. There was little difference between the varieties and by extracting under alkaline conditions and pptn. with HCl to pH 3·6—4·9, a product containing 62—75% of proteins was obtained. L. A. O'NEILL.

Respiration of Washington navel and Valencia oranges. S. A. Trout, J. E. Heulin and G. B. Tindale (*Commonw. sci. industr. Res. Org. Aust., Div. Fd Pres. Transp., tech. Paper*, 1960, No. 14, 11 pp.).—The respiratory drift of Washington navel and Valencia oranges from three coastal and four inland irrigated districts was measured after harvesting. In most cases a climacteric rise occurred as the oranges approached commercial maturity (**I**). Oranges picked a little before **I** are already in the climacteric phase and pass through a max. in respiration, while subsequent pickings start in the post-climacteric phase. Oranges affected by cold injury tended to give irregular curves. Oranges do not give the close correlation between the climacteric and other senescent changes characteristic of some other fruits. E. M. J.

Heat treatment of citrus for control of red scale. I. Trials on Washington navel fruit in a dehydrator. B. H. Martin and R. F. Black (*Aust. J. agric. Res.*, 1960, **11**, 197—207).—Oranges infested with red scale were placed in dehydrating ovens at temp. ranging from 50 to 70° and the insect mortality and fruit damage was recorded. At 50°, 100% "kills" were obtained in 2¼ hr. and no fruit was damaged. Raising the R.H. from 12 to 40% at 50° had no significant effect. S. G. AYERST.

Effect of sodium carbonate on black spot development in harvested citrus fruits. R. A. Christ (*S. Afr. J. agric. Sci.*, 1959, **2**, 575—577).—This disease is caused by the fungus *Phoma citricarpa*, McAl. By dipping the fruits in Na_2CO_3 solution (1—10% for 3, 15 and 30 min.) the condition of lemons was improved a little with a 5% solution, with a stronger solution some fruits were burnt severely. Navel oranges did not benefit from the dipping treatment and the keeping quality of Valencia oranges deteriorated. Na_2CO_3 solution does not kill the fungus within the fruit rind—and is only partially effective in control. I. DICKINSON.

Identification of pesticide residues in extracts of fruit, vegetables and animal fats. II. Rapid qualitative chemical tests for captan and methoxychlor. W. P. M Kinley and S. I. Graham (*J. Ass. off. agric. Chem. Wash.*, 1960, **43**, 89—91).—Captan is detected at the Canadian tolerance level by extracting a sample (900—1100 g.) with benzene for 30 min. in a roller stripper. A portion of the extract is dried, evaporated, dissolved in chloroform and added to a column of Florisil with washings in benzene containing 3% chloroform. The column is eluted with benzene containing 3% chloroform. Duplicate portions of the eluate are concentrated and placed on Whatman No. 1 paper which has been washed and dipped in 14% ethereal 2-phenoxyethanol. One spot is sprayed with $AgNO_3$ to detect all pesticides, and the other with a 1 : 1 mixture of $ZnCl_2$ and diphenylamine in acetone. The development of an intense green spot within 60 sec. of exposure to u.v. light confirms the presence of captan. Extracts are prepared similarly for methoxychlor. After the chromatography on Florisil, the eluate is analysed by the method of McKinley and Mahon, (*ibid.*, 1959, **42**, 725,) and confirmed by evaporating the eluate, adding fuming HNO_3 to the residue and after 30 sec. sodium methylate in methanol. A deep blue colour confirms the presence of methoxychlor. E. J. H. BIRCH.

Residues of *OO*-dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolothionate (Dimethoate) in sprayed crops. E. D. Chilwell and

P. T. Beecham (*J. Sci. Fd Agric.*, 1960, **11**, 400).—A method for determination of the residues is described. It was applicable to all the fresh vegetable and fruit crops analysed and the relationship of residue determined to toxic hazards to the consumer was studied by radiochemical and animal-feeding experiments. The analytical method gave a valid estimate of the total anticholinesterase hazard to the consumer and the limit of sensitivity is ~5 μg. of Dimethoate, equiv. to a residue of 0·1 p.p.m. in the plant tissue. Data on harvest residues in U.K. and overseas crops (e.g., 1—3 weeks after spraying) are presented. (12 references.) E. M. J.

Principles and instrumentation for the physical measurement of food quality with special reference to fruit and vegetable products. A. Kramer and B. A. Twigg (*Adv. Fd Res.*, 1959, **9**, 153—220).—General principles are discussed. Overall quality of a food is essentially a composite of several distinct attributes; the more completely and precisely a specific attribute is defined, the more probable is the attainment of a satisfactory instrumental method for its measurement. Quality measurements may be classified into categories of: appearance, kinesthetics (as felt by hand or mouth) and flavour. New techniques, e.g., ionising radiations, gas chromatography and nuclear magnetic resonance may be helpful in the development of instrumentation for direct measurement of flavour quality; a direct is preferable to a correlated method. (165 references.) E. M. J.

Chemistry and technology of preservation of green peas. L. J. Lynch, R. S. Mitchell and D. J. Casimir (*Adv. Fd Res.*, 1959, **9**, 61—151).—A detailed consideration of the chemical composition of peas forms the basis of this review and the effects of processing on the various constituents are discussed, the preservation of the nutritive value being of primary importance. Methods for grade or maturity determination, necessary for prediction of harvest date are discussed; all are compared with the final criterion, the taste test. For frozen and dehydrated peas, a younger optimal maturity than for canned peas is generally accepted. An outline of the harvesting, cleaning, grading and final processing is given. (212 references.) E. M. J.

Distinguishing between fresh and processed peas. L. Gersons and C. M. Swemle (*Ann. Falsif., Paris*, 1960, **53**, 144—159).—Determinations of % of retained water, alcohol-insol. solids and reducing substances on different types of fresh and soaked dried peas show no significant differences. Vitamin C and threonine contents are reduced in soaked dried peas. J. V. Russo.

Mono- and oligosaccharides of leguminous seeds and their behaviour during storage and germination. K. Täufel, K. J. Steinbach and E. Vogel (*Z. LebensmittUntersuch.*, 1960, **112**, 31—40).—Peas, beans and soya-beans contain <0·1% (dry basis) of glucose and fructose, and appreciable amounts of raffinose and stachyose; in addition, peas and beans contain, respectively, 0·9 and 0·15% of verbascose, and 2·5 and 1·6% of sucrose; the corresponding levels in soya-beans are traces and 8·1%. These levels remain constant during normal storage, but show small decreases in the verbascose and stachyose and moderate increases in the sucrose and raffinose when the seeds are stored in a warm and moist atm. Germination is accompanied by decreases in the higher oligosaccharides and increases in the sucrose content. Traces of galactose appear during abnormal storage or germination. Maltose (~0·3%) appears during the germination of soya-beans only. Interrupted germination brings about abnormal relations between the mono- and the oligosaccharides. The physiological significance of these findings is considered. (12 references.) P. S. Arup.

Determination of water-dispersible protein in soya-bean oil meals and flours. T. M. Paulsen, K. E. Holt and R. E. Anderson (*J. Amer. Oil Chem. Soc.*, 1960, **37**, 165—171).—An improved Waring Blendor method is described and compared with existing procedures. Effects of blending speed, temp., time, pH and design were studied. 20-g. samples were blended at 8500 r.p.m. for 10 min. at 25 ± 1° followed by aliquot centrifuging and nitrogen estimation. Results showed a high degree of accuracy and correlation with end-product uses for routine plant control. P. M. Kingston.

Reddening of white onion tissue. M. A. Joslyn and R. G. Peterson (*J. agric. Fd Chem.*, 1960, **8**, 72—76).—Addition of acetic acid to white onion purée was not necessary for the formation of the water-sol. red nitrogenous pigment, the acid's great enhancing effect being due to the 1 to 3 p.p.m. of formaldehyde that occurs in reagent glacial acetic acid as impurity. Similar compounds, such as diacetyl, acetoin and glycolonitrile, are as effective as formaldehyde in promoting the reddening. A type reaction mechanism for the pigment formation is postulated and discussed. The presence and absence in onions of several compounds are reported. (15 references.) S. C. Jolly.

Effect of low temperature on potato tubers. J. Fourré (*C. R. Acad. Sci., Paris*, 1960, **250**, 2261—2263).—Conductimetric values (*idem, ibid.*, 1958, **247**, 687) for potato tubers exposed for several hr. below 0° show that slow but effective destruction of tissue cells occurs at ~−3°, the proportion of dead cells increasing with decreasing temp. to −14°. Complete cell mortality occurs after ~13 hr., or after ~6 hr. if ice is brought into contact with the already frozen tissue. There is evidence of supercooling inside the tissues during the first 2 hr. and again between 4 and 6 hr. when the temp. is <~−5°. Frost resistance depends on variety and state of development of the tubers. W. J. Baker.

Changes in vitamin C content during kitchen treatment of potatoes. W. Franke (*Z. LebensmittUntersuch.*, 1960, **112**, 11—31).—The ascorbic and dehydroascorbic acid content of potatoes stored in a cool cellar decreases (according to the variety) from 15—35 mg. per 100 g. at harvesting time, to 5—15 mg. in April—May. Approx. % of culinary losses (based on the vitamin C content of the untreated tubers) are as follows: boiling in the skins or frying, ≯15; peeling followed by steaming or boiling (averages), 14—30 (subsequent mashing causes further losses of ~20%); baking, 50. The variations in the losses are very largely varietal. Peeled and quartered potatoes show considerable increases in vitamin C (max. ~70%) after keeping in a moist atm. at 6° during 48 hr., but the extra vitamin C thus obtained is subject to unusually high culinary losses. Keeping under water generally causes losses of vitamin C. Boiled or fried potatoes show considerable losses of vitamin C (max. 70% at room temp. or 34% at 6°) after keeping during 24—48 hr. (38 references.) P. S. Arup.

Rapid determination of sugar in potatoes in order to correct calculation of yields in potato starch manufacture. W. Maurer and E. Blaško (*Nahrung.*, 1959, **3**, 55—75).—A rapid method was developed in which the juice is centrifuged to remove solids, treated with a specially prepared resin and the sugar content of the residual liquor is then measured optically. The method of calculation used to obtain absolute values is outlined. The relative deviation of the results in comparison with other chemical methods is ±5% with a max. of 10%. A slightly more elaborate standard method based on the above procedure is also described. S. M. Marsh.

Determination of small amounts of arsenic in potatoes. Extraction and reduction of molybdoarsenic acid. D. J. Lisk (*J. agric. Fd Chem.*, 1960, **8**, 121—123).—In Wadelin and Mellin's modification of Akkseev's procedure for determining As, the As is measured as molybdoarsenic acid, which is extracted from the sample by butanol, after removal of interfering P as phosphomolybdic acid by extraction with 1-butanol/$CHCl_3$. The method is not quite sensitive enough for determining traces of As in biological materials. It is adapted for such use by acidifying the butanol extract with H_2SO_4/ethanol, and reducing to the heteropoly blue with $SnCl_2$ in ethanol. Interference by Si occurs if Si content is above 8 p.p.m., as in certain grasses; it can be lessened by using 1-butanol/ethyl acetate for extracting the molybdoarsenic acid. When the method was used to determine traces of As added to potatoes, average recovery was 94%. (10 references.) M. D. Anderson.

Effect of variety, curing and processing on carbohydrate content of precooked frozen sweet potatoes. M. W. Hoover and D. T. Pope (*Food Technol.*, 1960, **14**, 227—230).—The roots (cured and uncured) were sliced and cooked in syrup solutions containing 0, 30, 45 and 60% of sucrose by wt. The average % total sugar increased from approx. 9% for samples cooked in water for 10 min. to 21% for those cooked in a 60% sucrose solution, the increase being almost entirely sucrose from the cooking media. Wide variations occurred in certain carbohydrate components of the varieties (Porto Rico, Georgia Red, etc.), but each responded similarly during the cooking process. Curing treatments had little effect on the carbohydrates of frozen sweet potatoes. (12 references.) Wt. losses in cooking were >7% in some cases. E. M. J.

Effect of moisture content on storage of Brazil nuts. G. Ayerst and D. Budd (*J. Sci. Fd Agric.*, 1960, **11**, 390—396).—The collection, handling and storage of the nuts are described. The R.H., moisture content equilibrium, and the effect of moisture content on respiration rate were studied. Under storage conditions examined, there was little deterioration at moisture contents >~23% or <~11% but in the intermediate range, considerable deterioration occurred. These findings are discussed in relation to moisture contents of other seeds, and to storage of Brazil nuts in Brazil, transport by sea and storage, e.g., in England. (11 references.) E. M. J.

Proteins from deskinned groundnut kernels. J. P. Varma (*J. Oil Technol. Ass. India*, 1958 [1959], **14**, 21—23).—Prep. of white high-grade protein has been examined by treating the powdered (5 mesh) kernels with 0·2% NaOH or 10% NaCl followed by pptn. of the

protein with 5% acetic acid, 2·5% HCl or 95% alcohol at the isoelectric point. Yields and ash contents were higher for alkali-peptised than for salt-peptised protein. All experiments gave products of nitrogen content approx. 16%. P. M. KINGSTON.

Ethylene dibromide preparations and treating fruit therewith. N.V. de Bataafsche Petroleum Maats. (B.P. 816,202, 27.3.58. Isr., 29.3.57).—The compositions comprise 20—80% by wt. of ethylene dibromide, a petroleum distillate of b.p. 130—400° with low proportion of unsaturated and aromatic hydrocarbons, and at least one emulsifier capable of producing oil-in-water emulsions. E. ENOS JONES.

Non-alcoholic beverages

Micro-organisms in non-citrus juices. H. Lüthi (*Adv. Fd Res.*, 1959, **9**, 221—284).—Conditions in which microbial growth modifies fruit juice, problems involved in its prep. and storage and recent developments in this field are reviewed. Occurring bacteria, yeasts and moulds are listed, other organisms are mentioned and reference is made to their sources. Means of reduction of microbial count from infection in the fruit and/or lack of hygienic processing are detailed. Other conditions discussed are: changes in appearance of the juice, production of alcohols by micro-organisms (**I**), changes in org. acid contents and other changes induced by **I**. Dangerous thermoresistant moulds occur in large no. in some years, not in others. This and other unsolved problems are discussed. (229 references.) E. M. J.

Chemical constituents of citrus fruits. J. F. Kefford (*Adv. Fd Res.*, 1959, **9**, 285—372).—In this review of the literature for the period 1948/57 the general composition of citrus fruits is considered in great detail, including the extensive work on volatile and non-volatile flavouring constituents. (386 references.) E. M. J.

Adulterated concentrated orange juice. E. Benk (*Dtsch. Lebensmittrdsch.*, 1960, **56**, 99—103).—Analytical results on a series of commercial samples of conc. orange juice are discussed in relation to the results expected for a 6-fold concentrate of pure orange juice, and the results obtained on concentrated extracts of whole orange peel, and from extracts of the outer and inner peels separately. In some of the commercial samples high figures for extract, reducing and non-reducing sugars, and total acid, together with low protein, mineral matter, formol titre and phosphates clearly indicated additions of sugar and citric acid. In other samples unripe or damaged fruit had been used giving a product consisting wholly or in part of orange peel extract, and considered to be adulterated. Confirmation of such adulteration was not forthcoming from the manufacturers or suppliers; further investigation into the composition of natural orange juice from different varieties and producing countries is needed. (27 references.) E. C. APLING.

Detection of brominated oils in lemonades. E. Benk (*Riechstoffe u. Aromen*, 1960, **10**, 166—168).—For estimating the brominated oils the Carrez method of pptn. (by adding successively aq. K ferrocyanide and Zn acetate) is used; deriv. of monobromoacetic acid, if present, remain in solution for eventual detection and estimation. Before effecting the Carrez pptn. it is best (when small amounts of Br compounds are present) to ensure that iodine compounds are absent. (11 references.) H. L. WHITEHEAD.

Dehydrated orange juice. Vacu-Dry Co. (Inventor: W. R. Dorsey) (B.P. 815,354, 6.5.57).—Liquid orange juice concentrate is rapidly heated to 60—68·33° while simultaneously reducing the pressure as rapidly as possible to ≥ 8 mm., then after being kept thereat until thoroughly puffed the product is rapidly cooled to room temp. at reduced pressure, and ground to fine powder. This is admixed with a crushed mixture containing orange oil (and sorbitol) to provide a dehydrated orange juice. F. R. BASFORD.

Tea, coffee, cocoa

Variation in the nitrogen content of tea leaves. D. N. Barua and S. B. Deb (*J. Sci. Fd Agric.*, 1960, **11**, 366—370).—Data are presented showing the relationship of the N content of tea leaves to the level of N manuring, to the position in plucked and unplucked shoots and to genetic and environmental factors. The composition of a leaf varies with its position on the stem. For precision in sampling particular leaves, e.g., the second, of similar size, are chosen from equally sized shoots from bushes in the inside rows of a plot. E. M. J.

Roasting of coffee. C. P. Natarajan, R. Balakrishnan Nair, N. Gopalakrishna Rao, C. S. Viraktamath, D. S. Bhatia and A. N. Sankaran (*J. sci. industr. Res.*, 1960, **19A**, 32—37).—The roasting characteristics of *arabica* and *robusta* coffees were investigated to define the degree to which coffee beans had been roasted, in terms of finishing temp., colour, breaking strength and swelling ratio of the roast beans. Properties of each variety were plotted against colour of the coffee powder. To define the roasting of the coffee by its colour alone was insufficient; the relationship between temp., colour, breaking strength, swelling ratio and the ratio of caffeine to chlorogenic acid was preferable. A. ABBOT.

Production of heat during fermentation of cacao beans. R. H. Kenten and B. D. Powell (*J. Sci. Fd Agric.*, 1960, **11**, 396—400).—Heat produced during fermentation of the beans can arise from activities of micro-organisms on the pulp and from enzyme actions in the pulp, testa and cotyledons of the beans. Results of this study suggest that most of the heat is produced by the action of micro-organisms on the pulp. E. M. J.

Enzymic changes in cacao-bean during fermentation. I. Coupled reactions between the primary oxidation products of enzymic catechol-oxidation by cacao-polyphenolase, and proteins, peptides and amino-acids of cacao. A. Purr, R. Springer and H. Morcinek (*Z. Lebensmitt-Untersuch.*, 1960, **112**, 40—46).—Rates of O_2 absorption increase with the progress of germination, and show varietal differences in the initial and subsequent rates. The addition to the fermenting material, after the primary rapid stage of oxidation, of single peptides or amino-acids causes small increases in the (slower) secondary rates. In this respect, L-proline is, by itself, slightly effective, but very effective in combination with certain amino-acids or peptides, notably with glycine or glycylglycine. The other amino-acids occurring in the bean are either moderately effective or ineffective as accelerants in conjunction with L-proline. P. S. ARUP.

Estimation of fats and cellulose in cocoa products. H. Loft (*Mfg Confect.*, 1960, **40**, No. 2, 51—53).—A brief review. C. V.

Milk, Dairy Products, Eggs

Rapid determination of minerals and ions in milk. II. Chloride. V. R. Wenner (*J. Dairy Sci.*, 1960, **43**, 22—27).—A volumetric method involving direct titration with $Hg(NO_3)_2$ in HNO_3 solution and a potentiometric method using a Ag–AgCl electrode and a Pd–Hg calomel reference electrode are described for determining Cl^- in milk. A. H. CORNFIELD.

Influence of vacuum pasteurisation on the freezing-point value, total solids and concentration of fluid milk. J. T. Lazar, jun. and R. W. Henningson (*J. Dairy Sci.*, 1960, **43**, 42—47).—Concn. of milk did not occur in the Vacu-Therm pasteuriser until the temp. difference between the pasteuriser and the second vacuum chamber exceeded 11·1°. Both total solids values and f.p. measurements required correction for estimating water removal. A. H. CORNFIELD.

Size distribution of casein particles in cow, goat and human milk. E. Knoop and A. Wortmann (*Milchwissenschaft*, 1960, **15**, 273—281).—Electron microscopic examination showed the diameter of the casein particle was: human 42, cow 93 and goat (colostrum) 133 mμ. (20 references.) C. V.

Graduation of milk butyrometers. J. Pien (*Ann. Falsif., Paris*, 1960, **53**, 119—131).—Gravimetric and volumetric methods for the determination of fat in milk are compared and discussed with a view to providing a world-wide standard method. (20 references.) J. V. RUSSO.

An appraisal of the Gerber test for milk fat in milk and market milk products. D. Levowitz (*J. Milk. Tech.*, 1960, **23**, 69—72).—It is claimed that uniform, accurate results are obtained and that the test can be rapidly and simply carried out. (15 references.) C. V.

Inhibition of oxidised flavour in homogenised milk as related to the concentration of copper and phospholipids per unit of fat globule surface. N. P. Tarassuk and J. Koops (*J. Dairy Sci.*, 1960, **43**, 93—94).—Increasing the Cu concn. of the milk from 0·05 to 1·05 p.p.m. by addition of Cu before or after homogenisation increased the "oxidative flavour" score of the milk. The effect of Cu in increasing the score decreased with increasing homogenisation pressure. A. H. CORNFIELD.

Effect of heat on the chemical nature of the materials adsorbed on the milk fat globule. M. Loewenstein (*Dissert. Abstr.*, 1960, **20**, 3051—3052).—Material adsorbed on the surface of milk-fat globules was recovered by separating and washing the cream, churning it, extracting fat with light petroleum and concentrating and drying the remaining material. This material from whole milk momentarily warmed to 40° contained a mixture of normal milk lipids and proteins, which were not completely separated by the extraction. Milk subjected to heat treatment yielded less of this adsorbed material, and the content of protein and phospholipid was also decreased; part of the casein was replaced by other milk proteins.

The changes were more marked as heat treatment was intensified. Adsorbed material recovered from milk reconstituted by emulsifying milk fat in skim milk was generally higher in protein and lower in lipid than material from whole milk warmed to 40°. Heating the skim milk before emulsifying much increased the amount of the adsorbed material, which was mainly P-free lipid. In all cases, the lipid fraction of the adsorbed material contained S in amounts not explicable on the basis of known milk constituents.
M. D. ANDERSON.

Significance of strontium-90 in milk. II. Review of recent data and analysis of areas needing more elaboration to define the rôle of milk in the diet. B. L. Larson (*J. Dairy Sci.*, 1960, **43**, 1—21).—Recent data are summarised on the significance of ^{90}Sr and other radioactive fall-out in milk and other foods. The evidence indicates that people in primary milk-consuming areas are acquiring relatively lower levels of ^{90}Sr in their bones than people in primary plant-consuming areas. This is attributed to the high Ca level of milk which reduces bone accumulation of ^{90}Sr present in foods of plant origin in the human diet. For min. deposition of ^{90}Sr in bones it may be as wise to increase milk consumption as to try to remove ^{90}Sr from milk. A. H. CORNFIELD.

Removal of strontium from milk. L. Singer and W. D. Armstrong (*Nature, Lond.*, 1960, **186**, 484—485).—Approx. 60% of the ^{85}Sr can be removed by passing the milk (2 ml./min.) through a short column of powdered pretreated anorganic bone, without appreciable change in ionic composition or flavour. Pretreated fat-free bone removes 70—75% of Sr, but produces more change in ionic composition of the milk. Columns can be used continuously after regeneration with 4N-$CaCl_2$ (anorg. bone) or 4N-$CaCl_2$ followed by 8N-KOH (fat-free bone). Same solutions are used for pretreatment. W. J. BAKER.

Importance and detection of antibiotics in milk. W. Wodsak (*Nahrung*, 1959, **3**, 729—736).—The presence of antibiotics in milk leads to difficulties in processing and may have harmful effects on the consumer. Because of the small amounts usually present in milk, a combination of microbiological and chemical methods is most promising for the detection of antibiotics. Several such procedures are described and discussed. S. M. MARSH.

Adhesion-cohesion, static friction and macro-structure of certain butters. V. Friction and hesion measurements on the same test surfaces. J. W. Claassens (*S. Afr. J. agric. Sci.*, 1959, **2**, 551—571).—The relationship was studied between hesion (H) (stickiness) and apparent coeff. of static friction (μ') in commercial and experimental butters which were kneaded for varying length of time (10—50 min.). In the commercial butters a positive correlation ($r = 0{\cdot}76$) was found between H and μ'. Greatest hesion occurred in blended butters. In salted and in unsalted butters made in a constant temp. room, there was a positive relationship between μ' and H for the first 20 to 30 min. of kneading, after that a negative relationship occurred. (31 references.) I. DICKINSON.

Lipolytic deterioration of butter by micro-organisms. T. E. H. Downes (*S. Afr. J. agric. Sci.*, 1959, **2**, 527—541).—Cultures of *Candida lipolytica*, *Pseudomonas fluorescens* and an unidentified yellow mould which was isolated from a sample of rancid butter, were grown in sterilised skim-milk and inoculated into butter in quantities of 1%. The butter was then stored at 10, 32 and 56°F. Fat acid value, free volatile acid, free fatty acids (butyric, caproic, caprylic and capric), peroxide value, mould, yeast and bacterial counts, and organoleptic tests by a panel were determined at intervals. *C. lipolytica* was the most active of the three organisms at all three temp. Free butyric acid, not the only substance involved, had the greatest influence on the flavour; approx. 88 p.p.m. gave a rancid flavour. *C. lipolytica* produced this amount of butyric acid in 3 days at 56°F, in 7 days at 32°F and in 21 days at 10°F; *Ps. fluorescens* in 35—42 days, and the yellow mould in 28 days at 56°F, but not in 3 months at either 10 or 32°F. (10 references.) I. DICKINSON.

Continuous butter making. J. M. de Man and F. W. Wood (*Dairy Ind.*, 1960, **25**, 445—447).—Several processes exist; only in the U.S.S.R. is a substantial portion of the butter produced thus manufactured. The unsatisfactory consistency of the product is the most important factor preventing a more general acceptability. (10 references.) C. V.

Classification of the lactobacilli. M. Rogosa and M. E. Sharpe (*J. appl. Bact.*, 1959, **22**, 329—340).—A classification based on morphology, colony form, nature of fermentation, limiting temp. of growth, serology and physiological tests such as total acidity in milk, resistance to alkyl sulphates, production of gas from citrate and NH_3 from arginine, etc. is proposed. (44 references.) C. V.

Gas chromatography. II. Detection and determination of volatile fatty acids in dairy products and cultures. W. Ritter and H. Hänni (*Milchwissenschaft*, 1960, **15**, 296—302).—The carrier gas used was He and the fatty acids were obtained by steam distillation. *Staphylococcus aureus* and a micrococcus originating in the microflora of a Limburg cheese were used. In the following list, the source is indicated: formic acid (mainly from histidine), acetic acid (from most acids, chiefly alanine), propionic acid (α-aminobutyric acid and threonine), isobutyric acid (valine), n-butyric acid (norvaline), isovaleric acid (leucine), α-methylbutyric acid (isoleucine) and n-valeric acid (norleucine). (10 references.) C. V.

Determination of curd-making quality of non-fat dry milk. W. K. Stone, P. M. Large and G. C. Graf (*J. Dairy Sci.*, 1960, **43**, 48—53).—The curd-making quality of samples of non-fat dry milk was assessed by comparison with the product obtained from a control non-fat dry milk in laboratory cheese vats. There was a high correlation between the quality of cottage cheese made in the laboratory and in pilot size vats, but low correlation between curd-making quality and both whey protein-N and curd tension of non-fat dry milk. A. H. CORNFIELD.

Structure of Edam cheese. A. Fricker and G. F. Meyer (*Milchwissenschaft*, 1960, **15**, 281—286).—Examination by electron microscope revealed that the protein represents a uniform phase in which the fat globules are embedded. (11 references.) C. V.

Vitamin A_1 aldehyde in hen's eggs. P. A. Plack (*Nature, Lond.*, 1960, **186**, 234—235).—Vitamin A_1 aldehyde, previously found in ripe eggs of marine teleost fishes, was identified in hen's eggs by freeze-drying and powdering the yolk, extracting with ether, mixing the residue with aq. ethanol, extracting again with light petroleum, removing solvents and dissolving the extracts in *n*-hexane. On determining vitamin A_1 esters, alcohol and aldehyde, the ether extract contained 75 to 80% of the total lipids, and 95% of the free and esterified vitamin A_1, but no vitamin A_1 aldehyde. The light petroleum extract contained the remainder of the total lipids and free and esterified vitamin A_1, also vitamin A_1 aldehyde to the extent of about 22 μg. per egg (vitamin A_1 esters, 18 to 20 μg. per egg; vitamin A_1 alcohol 90 to 109 μg.). M. D. ANDERSON.

Egg food product. Gerber Products Co. (B.P. 818,003, 13.8.56).—Egg yolk is slurried (5—50%) with water, and the mixture is heated for 1—105 sec. at 88—147° (to cause partial coagulation of most of the protein), then rapidly cooled (to <60°, e.g., by flash evaporation), to give a stable, sterile egg yolk puree which is sealed in containers. F. R. BASFORD.

Edible Oils and Fats

Peroxide decomposition and carbonyl formation in autoxidised fats. K. Täufel and R. Zimmermann (*Ernährungsforschung*, 1960, **5**, 104—109).—Autoxidised fats (sunflower oil, hardened soya-bean oil, or lard) containing $CuCl_2$ or $FeCl_3$ (in concn. 10^{-3}M) show, after heating in closed vessels at 100° during 1 hr., decreases in peroxide value to $\not> 1$ and proportionate increases in saturated and 1,2-unsaturated aldehydes. The aldehydes are collectively detected in steam distillates from the fats by the yellow coloration formed on heating at 70° during 30 min. with a reagent containing thiobarbituric acid and $FeCl_3$, and determined by spectrophotometric measurement at 452 mμ. Results obtained with the usual (thiobarbituric acid) test for malondialdehyde show no connexion with the decreases in peroxide value. P. S. ARUP.

Method for studying influence of metal salts on rancidity of fats. R. Vásquez Ladrón, R. Guriérrez González-Quijano and J. M. R. de la Borbolla y Alcalá (*Grasas y Aceites*, 1960, **11**, 26—28).—Filter paper is impregnated with the metal salt solution, and after drying, with the oil. The development of rancidity on storage is followed organoleptically or from the peroxide value. Metals having the most pronounced catalytic influence were Cu, Co, Mn, Fe and Tl.
L. A. O'NEILL.

Toxicity of air-oxidised soya-bean oil. J. S. Andrews, W. H. Griffith, J. F. Mead and R. A. Stein (*J. Nutr.*, 1960, **70**, 199—210).—The toxic principle corresponded closely to the peroxide concn. of the oil. It is suggested that intestinal xanthine oxidase is inhibited. (24 references.) C. V.

***Trans* fatty acid content of margarines and shortenings.** A. F. Mabrouk (*Dissert. Abstr.*, 1960, **20**, 3052—3055).—The i.r. spectrophotometric method of Swern *et al.* for determining *trans* components in hydrogenated fats was applied to six margarines and five shortenings. New i.r. reference values were determined for several pure acids and esters. *Trans* acids formed 33 to 42% of the margarines, and 23 to 37% of the shortenings, except the blending shortening Swiftning, which contained only traces; it was the only

product to contain arachidonic acid (0·45%). Cottonseed, soya-bean and groundnut oils, and maize and lettuce-seed oils, contained no *trans* glycerides. *Trans* glycerides in coconut, palm and olive oils were 2·6, 2·3 and 9·4%, and in lard, human milk fat and human body fat, 0·0, 7·3 and 3·0%. Conversion to methyl esters did not affect the constituent acids; fractional distillation of the esters showed *trans* components in all fractions, with 40 to 50% in the C_{18} esters. The unsaturated fatty acids of margarines and shortenings are extremely complex mixtures of many of the theoretically possible isomers of the octadecenoic and octadecadienoic acids. The limitations of i.r. and u.v. spectrophotometric methods are evaluated.
M. D. ANDERSON.

Utilisation of spent nickel catalyst from vanaspati industry. O. Prakash, Atma Ram and J. Chandra Gupta (*J. Oil Technol. Ass. India*, 1959, **14**, 24—32).—Different methods of treating the nickel waste have been examined. The best procedure involves heating with formic acid for several hr. at 105° followed by fat extraction with 60—80° light petroleum. The resulting Ni formate solution is concentrated, and treated with H_2O_2 and basic Ni carbonate to precipitate impurities. Recovery of 87—97% nickel having good catalytic activity is reported. (13 references.)
P. M. KINGSTON.

Utilisation of by-products from vanaspati industry. R. K. Bhatnagar and B. G. Sharma (*J. Oil Technol. Ass. India*, 1958 [1959], **14**, 50—52).—A discussion on industrial uses of cottonseed-oil soap stock, filter-press mud and spent nickel catalyst.
P. M. KINGSTON.

Chemicals from castor oil. A. S. Gupta (*J. Oil Technol. Ass. India*, 1959, **14**, 47—49).—The industrial application of undecylenic acid, heptaldehyde, sebacic acid and 2-octanol are reviewed.
P. M. KINGSTON.

Mixed triglycerides. T. Hedley & Co. Ltd. (B.P. 816,343—4, 9.4.56. U.S., [A] 7, [B] 8.4.55).—A glyceride oil, suitable for use as a salad oil or for prep. of whole-egg mayonnaise comprises a mixture of glycerides from (*a*) acetic, propionic, butyric or caproic acids, and (*b*) a high-mol.-wt. fatty acid of 8—24 C-atoms derived from an animal fat, a marine oil or a vegetable oil of the oleic–linoleic acid group or hydrogenated products thereof, having an I val. [A] >80, [B] >55, [A] the molar ratio of (*a*) : (*b*) being 1 : 4—2 : 1. In [B] there may be present instead of (*b*) a high-mol.-wt. fatty acid of the lauric acid group or a hydrogenation product thereof. One example is [B] hydrogenated coconut oil of I val. 1·0 is inter-esterified with triacetin.
E. ENOS JONES.

Edible fat. J. Bibby & Sons Ltd. (Inventors: R. V. Crawford and H. Jasperson) (B.P. 816,514, 9.7.56).—An edible fat useful as a substitute for palm kernel or coconut oil stearine, or as a "hard" butter, is obtained by subjecting palm kernel oil, alone or admixed with ⩾ 10% by wt. of another oil rich in 16—18-C acids (e.g., palm oil) to a mol. rearrangement at a temp. above the m.p. of the fat combined with a hydrogenation process.
E. ENOS JONES.

Meat and Poultry

Chemical changes associated with the ageing of meat with emphasis on proteins. J. R. Whitaker (*Adv. Fd Res.*, 1959, **9**, 1—60).—This review covers the structure of skeletal muscle, proteins of muscle, chemical changes associated with contraction and with onset of rigor mortis, rigor mortis and thaw rigor and chemical changes associated therewith, artificial "ageing" of meat and commercial tenderisers. The present state of knowledge is summarised, unsolved problems are discussed and the application of the techniques of electrophoresis, ultracentrifugation, chromatography and solubility is suggested. (286 references.)
E. M. J.

Food grinder-recording ammeter method for measuring beef tenderness. J. A. Emerson and A. Z. Palmer (*Food Technol.*, 1960, **14**, 214—216).—In trials comparing the Warner–Bratzler shear apparatus (**I**) and the food grinder (**II**) methods with that of a taste panel (**III**), **III** was the most repeatable method followed by **I** and **II**, using broiled samples. **I** is a more precise measurement of tenderness than **II**.
E. M. J.

Correlations between the pH value of meat and the diffusion of salt. L. Körmendy and G. Gantner (*J. Sci. Fd Agric.*, 1960, **11**, 377—380).—The speed of penetration of salt into loin pork meat under constant conditions of pickling (102 samples of 10-cm. cylinders, 122—200 g. wt.; ratio of pickle to meat; temp.) was studied and the results were statistically analysed. The salt concn. of the meat after 24 hr. was considered a good approx. measure of speed of diffusion. There was no close correlation between pH of the meat and speed of salt penetration. The samples tested had pH within narrow range (87% between pH 5·4 and 6·3 and none between 6·8 and 7·0).
E. M. J.

The sodium and potassium of bone mineral. T. A. Taylor (*Experientia*, 1960, **16**, 109—110).—Exhaustive acid extraction, by fractionation, of bone powders, indicated almost total and relatively easy solubility of the K in dil. acid, while even substantial amounts of Na remained in the bone powder, after vigorous treatment with acid, indicating that K lies almost totally in the hydration layer, and that the Na is present in two forms—one relatively sol., and one relatively insol.—a conclusion similar to that reached for the Mg in the bone. (In English.)
M. LAPIDOT.

Moisture levels in frozen poultry as related to thawing losses, cooking losses and palatability. I. Chicken broilers. G. W. Froning, M. H. Swanson and H. N. Benson (*Poultry Sci.*, 1960, **39**, 373—377).—A large proportion of the moisture absorbed in processing and liquid-chilling chicken broilers was lost on thawing and additional losses occurred on cooking. Flavour scores of roasted birds were adversely affected where they had been subjected to prolonged chilling. Juiciness was not satisfactorily improved by addition of moisture during processing.
A. H. CORNFIELD.

Micro-organisms from chicken meat related to both lactobacilli and aerobic spore formers. M. J. Thornley and M. E. Sharpe (*J. appl. Bact.*, 1960, **22**, 368—376).—Certain Gram-positive, catalase-negative, rods with a homofermentative action on glucose were isolated from chicken meat; these resembled lactobacilli in many respects but showed reluctance to grow on the normally favourable medium. No spores were formed and three groups were noted: (*a*) rod-shaped or filamentous, non-motile with no action on litmus milk (**I**), arginine (**II**) or aesculin (**III**); (*b*) similar to (*a*) but **I** was reduced and **II** and **III** were hydrolysed while (*c*) were short motile rods with an effect on **I, II** and **III** similar to that of group (*b*). (23 references.)
C. V.

Treating meat. Swift & Co. (B.P. 818,175, 14.2.56. U.S., 24.2.55).—Meat, especially sausage, is cured by bringing it into contact with NO under such conditions as to impart a cured colour without causing it to become green. The NO may be used as an aq. solution in which the meat is immersed, or it may be introduced (at reduced pressure) into an aq. emulsion of the meat. A further method of treatment comprises chopping the meat with ice containing NO until the ice melts and an emulsion is formed.
F. R. BASFORD.

Fish

Amino-acid composition of herring (*Clupea harengus*) and herring meal. Destruction of amino-acids during processing. G. Boge (*J. Sci. Fd Agric.*, 1960, **11**, 362—365).—Studies by microbiological methods are reported and values found for herring meal are in agreement with earlier published data. No destruction seems to occur during processing conditions described. Temp. and time of drying are important. Examination of herring press water and condensed herring solubles shows that destruction of certain amino-acids may occur during evaporation; cystine is nearly destroyed, losses of histidine and tryptophan amount to 43 and 36% respectively. Spontaneous heating of the meal causes losses of all amino-acids and especially of lysine, tryptophan, cystine and histidine. (10 references.)
E. M. J.

Penetration of tetracycline antibiotics into tuna, sole and rockfish flesh and their stability during steaming and retorting. P. A. Lerke and L. Farber (*Food Technol.*, 1960, **14**, 217—221).—The skin and outer layer of tuna flesh prevented the penetration of the antibiotic (**I**) into the deeper layers of the meat. **I** was then more easily destroyed by the subsequent steaming process in the tuna canning procedure. The uptake of chlortetracycline (**II**) by sole fillets was rapid during the first 5 min. of immersion, then decreased up to 1 hr. **II** was stable in sole and rockfish fillets up to 7—9 days at 41°F and average losses during storage were 16·8 and 16·5% respectively. The amounts of **I** remaining after steaming, baking or frying were relatively insignificant. (19 references.)
E. M. J.

Biochemical investigations on quality classification of fish. F. Bramstedt and I. Worzbacher (*Fette Seif. Anstrichm.*, 1960, **62**, 513—517).—By determining the free amino-acid content of fish muscle by standard methods, on fish of known organoleptic quality, it is shown that it is possible to characterise such fish quality by the free amino-acid content. Shellfish have been similarly classified. Amino-acids so determined are: histidine, lysine, leucine and isoleucine and the total amino-**N**. (15 references.)
G. R. WHALLEY.

Prevention of struvite formation in thermally treated seafoods. Blue Channel Corp. (B.P. 817,088, 27.1.58. U.S., 11.2. and 24.4.57).—Struvite formation during the thermal treatment and canning of seafoods (shrimp, crayfish, lobsters, etc.) is prevented by adding to

the can (containing the seafood and brine or water) 0·025—0·2 wt.-% of $Al_2(SO_4)_3,18H_2O$ as a solution, powder or tablet).
F. R. BASFORD.

Preservation of proteinaceous foodstuffs. F. E. de Vries (B.P. 815,465, 2.12.54).—Proteinaceous foodstuff of high moisture and protein content, e.g., meat and fish, is preserved by mixing the comminuted material with 40—85 wt.-% of starch or starch-containing flour, then drying (at 50—55°) to <15 (10—15) wt.-% of moisture. If desired, either prior to or after the mixing process. oil (especially herring oil) may be recovered from the foodstuff after treatment with warm air (e.g., at 80°). F. R. BASFORD.

Spices, Flavours, etc.

Vanilla extract. II. Stability of lead test solution. W. J. Considine and B. Pearl (*Food Technol.*, 1960, **14**, 204).—Using an ethylenediaminetetra-acetic acid (EDTA) titration method for the determination of Pb, triplicate blank determinations, according to the official method (A.O.A.C.) were run on a Pb test solution. Freshly prepared solution had a Pb content of 0·229, 0·229 and 0·0230 g./100 ml., and after storage for 10 days: 0·229, 0·229 and 0·229 g./100 ml. This and data for two other Pb tests show that such solutions can be stored for at least 1 week without any significant fall in titre. Only one blank will suffice for a series of Pb no. determinations. E. M. J.

Methods for the analysis of vanilla extracts for resins, carbonyl compounds, amino-acids and other organic acids. E. A. Prill, C. A. Porter, R. C. Staples and H. P. Burchfield (*J. Ass. off. agric. Chem. Wash.*, 1960, **43**, 96—107).—A systematic analysis is described of genuine and adulterated or synthetic vanilla extracts and a comparison of the results given. Gums are precipitated by increasing the alcohol content, centrifuging and weighing, and resins by evaporating to collect volatile material, adjusting the residue to pH 2 and centrifuging. The proportion of these resins sol. in tetrahydrofuran, methanol and aq. NH_3 respectively are also determined gravimetrically. The i.r. spectra of the resins are determined by evaporating them from a tetrahydrofuran solution on to a rock salt window. Carbonyl compounds are determined in the resin-free filtrates gravimetrically with 2,4-dinitrophenylhydrazine, Amino-acids and other org. acids are separated by absorption of the amino-acids on a cation-exchange resin in the H^+ form. The amino-acids can be determined colorimetrically (with ninhydrin) as total amino-acid or separated by 2-dimensional paper chromatography. The other org. acids are characterised by the elution pattern produced on gradient elution from an anion-exchange resin (in the formate form) with formic acid. Authentic extracts at single strength have a gum content of 52 to 160 mg. ml. and a resin content of 57 to 144 mg./ml. Most of the crude resin is sol. in tetrahydrofuran. The i.r. spectrum of the resin has 11 main peaks (detailed). The i.r. spectra of adulterated and synthetic extracts are sufficiently different to arouse suspicion. The spectra of possible adulterants such as St. John's-wort and polymerised vanillin are shown. The org. and amino-acid contents are discussed but it is suggested that many analyses of authentic extracts are needed to establish the normal range of variability. E. J. H. BIRCH.

Quantitative oscillographic determination of vanillin and bourbonal in vanilla sugar pudding and sauce mixes. H. Woggon and K. Rauscher (*Nahrung*, 1959, **3**, 161—173).—Methods available for the determination of vanillin are briefly reviewed. Details of an oscillographic procedure are given with circuit diagrams in which semicarbazide hydrochloride is used as electrolyte. The method is suitable for qual. routine control, the main advantage being that no preliminary treatment of the sample is necessary.
S. M. MARSH.

Chemistry and technology of garlic powder. J. S. Pruthi, G. Lal and V. Subrahmanyan (*Food Sci. Mysore*, 1959, **8**, 429—431).—A brief introductory review of the manufacture and application of garlic powder. (24 references.) P. M. KINGSTON.

Determination of the critical temperature of dehydration of garlic. J. S. Pruthi, L. J. Singh and G. Lal (*Food Sci. Mysore*, 1959, **8**, 436—440).—The effect of temp. (30—70°) after 4 and 8 hr. intervals on fresh garlic powder was studied in respect of moisture content, allicin, allyl sulphide and antibacterial activity. Deterioration was appreciable above 60°; loss of allyl sulphide and antibacterial activity in fresh garlic occurred above 50° especially after 8 hr. exposure. This change is reflected in colour and flavour breakdown. (13 references.) P. M. KINGSTON.

Technological aspects of dehydration of garlic: factors affecting the quality of garlic powder during dehydration. J. S. Pruthi, L. J. Singh and G. Lal (*Food Sci. Mysore*, 1959, **8**, 441—444).—Attempts to improve antibacterial activity, flavour and colour during dehydration by ascorbic acid spraying (200 mg./100 g.), sulphuring and sulphitation have proved unsuccessful. Variation of tray-loading density indicates an optimum at 1·14-lb. fresh garlic per sq. ft. tray surface. (20 references.) P. M. KINGSTON.

Effect of different methods of dehydration on the quality of garlic powder. J. S. Pruthi, L. J. Singh, S. S. Kalbag and G. Lal (*Food Sci. Mysore*, 1959, **8**, 444—448).—The product from freeze drying and vac.-shelf drying shows a slightly better colour than from hot-air drying but other characteristics are not improved. Through-flow drying gives a 50% reduction in time over cross-flow drying.
P. M. KINGSTON.

Pilot plant studies on the manufacture of garlic powder. J. S. Pruthi, L. J. Singh, S. D. V. Ramu and G. Lal (*Food Sci. Mysore*, 1959, **8**, 448—452).—Using the cross-flow drying method, 26% yields (6% moisture) were obtained at 30% lower cost and better quality than by older techniques. Moisture uptake of the powder was significant at 20% R.H. but with chocolate- or sugar-coated tablets more than 50% R.H. is required. P. M. KINGSTON.

Effect of type of packaging and storage temperature on allyl sulphide, total sulphur, antibacterial activity and volatile reducing substances in garlic powder. L. J. Singh, J. S. Pruthi, V. Sreenivasamurthy, M. Swaminathan and V. Subrahmanyan (*Food Sci. Mysore*, 1959, **8**, 453—457).—Garlic was stored at 37°, room temp. and 0—2° for 10 months in glass bottles, sealed tins, polythene bags, gelatin capsules and in the form of tablets. Least deterioration occurred in the tins and at the lower temp. P. M. KINGSTON.

Effect of type of packaging and storage temperature on flavour and colour of garlic powder. L. J. Singh, J. S. Pruthi, A. N. Sankaran, K. Indiramma and G. Lal (*Food Sci. Mysore*, 1959, **8**, 457—461).—Storage tests for 6, 12, 20 and 40 weeks at 37°, room temp. and 0—2° were carried out in glass bottles, sealed tins, polythene bags and gelatin capsules. Whereas polythene bags showed max. flavour loss, garlic powder of low moisture (6%) was relatively stable in bottles and sealed cans, especially at lower. temp.
P. M. KINGSTON.

Effect of nitrogen packing and storage temperature on the quality of garlic powder. J. S. Pruthi, L. J. Singh, K. Indiramma, A. N. Sankaran and G. Lal (*Food Sci. Mysore*, 1959, **8**, 461—464).—In 6 weeks' storage tests at 37°, room temp. and 0—2° in white and brown bottles, the use of N_2 showed little advantage in respect to antibacterial activity and organoleptic changes. Losses of allyl sulphide are slightly reduced by N_2 especially at the lower storage temp. P. M. KINGSTON.

Salt crystals. Imperial Chemical Industries Ltd. (Inventors: C. Allday and T. N. Belford) (B.P. 818,385, 4.11.55).—A substance, which in aq. solution gives rise to multivalent complex anions (especially a cobalticyanide or a ferricyanide deriv.), is added to solid salt to reduce its tendency to cake. J. M. JACOBS.

Preservatives

Diphenyl and *o*-phenylphenol as preservatives for citrus fruit: a simple method for detection and determination of the *o*-phenylphenol. M. Ihloff and M. Kalitzki (*Dtsch. LebensmittRdsch.*, 1960, **56**, 139—140).—The method consists in a distillation of the disintegrated fruit, mixed with water, phosphoric acid and a little anti-foam, in a special return-flow apparatus, the ethereal oil collected containing all the *o*-phenylphenol. An aliquot of the oil is extracted from light petroleum with 0·1N-NaOH, and aliquots of the alkaline solution are chromatographed on paper with 0·1N-NaOH as mobile phase. Spots are identified and estimations made by spraying with an alcoholic solution of 2,6-dibromoquinonechlorimide and comparison of the R_F value with controls of known amounts of *o*-phenylphenol. Results within about 20% of the actual are reported. C. L. HINTON.

Rapid spectrophotometric method for simultaneous determination of benzoic and sorbic acids in margarine and butter. J. B. Roos and A. Versnel (*Dtsch. LebensmittRdsch.*, 1960, **56**, 128—133).—The method described depends upon the extraction of the benzoic and sorbic acids from 5 g. of the warmed margarine or butter with an acidified mixture of methanol and water. After cooling and solidification of the fat the methanolic solution is filtered and the absorption is measured at 228 mμ (for benzoic acid) and 258 mμ (for sorbic acid), and also at the isosbestic wavelength 233·3 mμ. Several methods of calculating results are given, for cases where the single or both acids are present and where it is desired to correct for unspecific general absorptions and for traces of fats which are extracted by the solvent. Recoveries usually within 50 p.p.m. are reported. (26 references.) C. L. HINTON.

Salicylates. Monsanto Chemicals Ltd. (Inventor: A. M. Spivey (B.P. 816,245, 25.10.56).—A salicylyl halide (or a substituted derivative thereof) is condensed with ascorbic acid in presence of a H halide acceptor (pyridine), to give *ascorbyl trisalicylate*, m.p. 81—85° (or a derivative thereof). The compounds and their salts (e.g., Na salt) are useful as combined fat antioxidants and food preservatives. F. R. BASFORD.

Food Processing, Refrigeration

[A] Convection in syrup-packed products. [B] Product-induced stratification of covering syrups. [C] Convection heating studies of water–syrup layered systems. R. C. Nicholas, I. J. Pflug and T. R. Mulvaney (*Food Technol.*, 1960, **14**, 205—207, 207—211, 212—214).—[A] Combinations of syrup (sucrose) concn. and temp. that will prevent convection currents on heating syrup-packed products are discussed and a method of calculating a criterion of no convection is presented. (16 references.)

[B] Dynamic processes which result in product-induced stratification in sweet fresh cucumber spears are described. During heat processing of sugar-packed products the water leaving the product rises to the top, dilutes the syrup (sugar and acid) and conditions to which micro-organisms are exposed are changed. In some regions of the jar the sugar and acid conditions may be significantly lower than were anticipated when the heat process was designed.

[C] Two model systems with built-in stratification that restricted convection to selected regions of a jar, were examined to determine the effect of stratification on the heating characteristics throughout the jar. Although the resemblance to food systems is remote the results are suggestive of the changes in heating characteristics that are brought about by product-induced gradients, and density gradients produced by, e.g., addition of dry sugar. E. M. J.

Cold storage and freezing of foodstuffs. W. Schweisheime, (*Riechstoffe u. Aromen*, 1960, **10**, 176—179).—A review. H. L. WHITEHEAD.

Production of carbon dioxide and dry ice. K. Nurmberger and H. Kubli (*Mod. Refrig.*, 1960, **63**, 302, 305—306, 309; 404, 407—408, 411—415).—A review. C. V.

Deep-freezing of bread. T. Kouwenhoven (*Bakkerswereld* 1959/60, **20**, Repr. 7 pp.).—Bread can be kept in fresh condition at −30° during 3 days, but as the texture deteriorates more rapidly within a fairly wide range of temp. centring round −2° than at higher or lower temp., it is essential that both cooling and thawing be conducted as rapidly as possible. Satisfactory results are obtained by cooling moderate-sized loaves by means of circulating air at −30°, and by thawing at 60°. P. S. ARUP.

Packaging

Pattern of redness loss and regeneration in prepackaged beef. R. W. Dean and C. Olin Ball (*Food Technol.*, 1960, **14**, 222—227).—Beef cuts vac.-packed in cans or film packages discolour on top and bottom surfaces during the first day of storage, but in materials of low permeability to air, undergo a return to redness on the second, third or fourth day of storage, and the colour is relatively stable. Small cans, Al foil, polyethylene and polyethylene coated with polyvinyl alcohol were the three most satisfactory materials used. The pattern of colour change is sometimes identical in beef cuts from different animals, but there is evidence that different inherent characteristics of meat from different animals may be responsible for substantial differences in colour history during storage. E. M. J.

Sterilising process. Deutsche Gold-u. Silber-Scheideanstalt (B.P. 818,663, 25.10.57. Ger., 30.11.56).—Sterilisation of all types of articles, especially foodstuffs and plastic packages is effected by treatment with a mixture of ethylene oxide (25—75) and propylene oxide (75—25%). F. R. BASFORD.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Pulse proteins: association with thiamine and nicotinic acid content of pulses. G. C. Esh and T. S. De (*J. Inst. Chem. India*, 1960, **32**, 14—16).—Thiamine (**I**) and nicotinic acid (**II**) contents of low- and high-protein pulses of different varieties, strains and localities were determined. Strains yielding high protein contents may not give high yields of **I** and/or **II** and vice versa. J. V. RUSSO.

Proteins of double bean (*Faba vulgaris* Moench). I. Isolation, fractionation and amino-acid composition of proteins. II. *In vitro* digestion. Kunjlata Kothary and Kamala Sohonie (*J. sci. industr. Res.*, 1960, **19C**, 14—16; 16—19).—I. Three proteins were isolated from double beans by $(NH_4)_2SO_4$ fractionation of an aq. extract of bean meal. N distribution in these proteins was studied by the van Slyke method, using casein as a standard reference protein. A microbiological assay of the essential amino-acids, using the Barton-Wright procedure, showed that the proteins are poor in leucine, isoleucine, valine and methionine but rich in arginine, lysine and threonine compared with casein. Proximate analyses of the proteins are also given. (16 references.)

II. *In vitro* digestibility tests on the double bean proteins by: (i) pepsin, (ii) trypsin and (iii) pepsin followed by trypsin are reported. The Kunitz spectrophotometric method was used to measure the extent of hydrolysis. In (i) all three proteins were almost completely digested within 4 hr., but were much less digestible than casein. In (ii), the proteins were found to be associated with a trypsin inhibitor, but in (iii) the inhibition was not noticed and there was very little difference between these proteins and casein. A. ABBOT.

Colorimetric determination, without previous isolation, of glutamic acid in hydrolysates of nutrients. H. Zimmermann (*Z. Lebensmitt-Untersuch.*, 1960, **112**, 46—49).—The application of the method (described) used by Sachs and Brand (cf. *J. Amer. chem. Soc.*, 1954, **76**, 3601) to various amino-acid mixtures with or without glutamic acid (**I**) is examined. The extinction values (E) for mixtures of animal origin without **I** are sufficiently uniform and (in comparison with the values given by **I**) small to enable the determination of **I** (within ±2% for test solutions containing 15—20 mg. of amino-acids) by means of a linear graph from $E = 0{\cdot}033$ for 0% to $E = 0{\cdot}55$ for 100% of **I**, the concn. of the acids being determined as N × 6·25. The results obtained for plant hydrolysates are subject to a constant deduction of 0·6% (total N × 6·25 = 100%) to allow for the coloration given by γ-aminobutyric acid. Allowance must also be made for the possible presence of substances in the samples giving a coloration with hydroxylamine and $FeCl_3$ without previous treatment with HNO_2. Formulae for the calculation of results are also given. (10 references.) P. S. ARUP.

Photometric estimation of colour reactions on paper. Simple procedure for quantitative amino-acid determination. K. H. Menke. (*Z. anal. Chem.*, 1960, **172**, 423—428).—The method is valuable for determination of amino-acids separated by column chromatography. After reaction of the acids on paper with ninhydrin reagent, the transmittancy is measured at 630 and 530 mμ; this eliminates error due to non-uniformity of the paper. A conversion table of colour readings into amounts of various amino-acids is given; sensitivity is 0·2 μg. of leucine per ml. and the standard deviation for 30 μg. per ml. is ±0·83 μg. (150 determinations.) P. D. PARR-RICHARD.

Turbidimetric and titrimetric methods for the microbiological determination of amino-acids: a unification of materials. J. J. McGuire, S. S. Schiaffino and H. W. Loy (*J. Ass. off. agric. Chem., Wash.*, 1960, **43**, 34—37).—The necessity of using a titrimetric method for the microbiological assay of amino-acids, instead of the shorter turbidimetric method, when colour or extraneous turbidity interferes, makes it desirable to standardise a medium and an organism for each amino-acid that is suitable for both. A formulation of a stock solution for the medium is given, and calibration curves are given for both titrimetric and turbidimetric assays of 12 amino-acids using three micrro-organisms. E. J. H. BIRCH.

Paper-chromatographic analysis of acids (horizontal migration method). VI. Separation and identification of amino-acid mixtures. V. K. Mohan Rao (*J. sci. industr. Res.*, 1960, **19B**, 62—66).—The paper chromatographic behaviour of amino-acids in the presence of halogen acids and salts was studied. The R_F of amino-acids are influenced by the nature and concn. of the salt in the solvent rather than in the solution to be chromatographed. Presence of salt prevents the streaking of arginine and lysine halogen acid salts and lowers the R_F values of amino-acids at higher concn. (23 references.) O. M. WHITTON.

Determination of cystine by catalytic effect on iodine-azide reaction. R. D. Strickland, P. A. Mack and W. A. Childs (*Analyt. Chem.*, 1960, **32**, 430—436).—This simple and accurate colorimetric method for determination of μg. quantities of cystine in protein hydrolysates is based on its catalytic effect on the reduction of iodine to iodide by Na azide. The method may find application for estimating other substances containing bivalent S. A. R. ROGERS.

Symplex formation between thermally oxidised oil and protein. M. Sugai (*Dissert. Abstr.*, 1960, **20**, 3257).—The formation of a complex (symplex) between heat-oxidised maize oil and protein was affected by the no. of CO, OH, and epoxide groups, and the no.

and position of double bonds in the oil. Least symplex was formed when COOH groups were blocked. Peroxide groups were apparently not involved in symplex formation. Not all proteins form symplexes with heat-oxidised maize oil; the active centres in proteins appear to be the COOH groups. Pancreatic lipase liberated fatty acids from a symplex of heat-oxidised maize oil and egg albumin, but could not completely hydrolyse the maize oil *in vitro*. Lipid could not be removed from the symplex by boiling org. solvent, or treatment with less than 10% alkali. Proteolytic hydrolysis with enzymes occurred only after removal of lipid by lipolytic enzyme. When symplex was fed to rats, excretion of protein in the faeces was much increased; similarly when heat-oxidised maize oil was fed, so that symplex may be formed in the gastrointestinal tract. Up to 20% of the lipid of heat-oxidised maize oil or symplex was not absorbed. M. D. ANDERSON.

Comparative feeding value of common edible fats. E. W. Crampton, R. K. Shaw, V. G. Mackay and D. C. Schad (*J. Nutr.*, 1960, **70,** 81—90).—Prematurely weaned puppies (108), pigs (172) and guinea pigs (266) were studied; butter fat was compared with 15 other edible animal and vegetable fats and oils fed as a 20% by wt. of the ration. Growth, voluntary feed intake, apparent digestibility and gain per 1000 digested kg.-cal. were measured. Some preference was shown for rations containing butter but no significant difference could otherwise be detected between any of the substances used; these included, wheat (**I**), soya-bean (**II**), cottonseed (**III**) and coconut (**IV**) oils, hydrogenated **I**—**IV** products, lard, mixed tallow and beef. (31 references.) C. V.

Fat metabolism. K. Täufel (*Ernährungsforschung*, 1960, **5,** 95—103).—A review covering physiological considerations arising from recent investigations on the composition, assimilation and selective distribution (between the blood and lymph systems) of fats. (13 references.) P. S. ARUP.

Effects of diet on fish oil toxicity in the rat. B. H. Ershoff (*J. Nutr.*, 1960, **71,** 45—53).—Immature rats fed on a purified low fat diet supplemented with 10% fish oil (tuna, sardine, menhaden or cod-liver) showed retarded growth and diarrhoea. These symptons were counteracted by concurrent administration of cottonseed, soya-bean, sesame (**I**), maize and wheat germ oils (15%) but olive and coconut oils and butterfat, lard and hydrogenated cottonseed oil possessed little or no protective effect. Melinoleate (1·5 or 10%) and α-tocopherol (**II**) acetate (0·025—0·1%) were also ineffective. Administration of **II** (0·025—0·1%) gave a degree of protection but to a lesser extent than *NN'*-diphenyl-*p*-phenylenediamine or 6-ethoxy-2,2,4-trimethyl-1,2-dihydroxyquinoline (Santoquin) fed at 0·05%. The methylene ether of oxyhydroquinone (sesamol), which is present in **I** was also effective (0·05 and 0·017%) and this possesses an antioxidant effect. Lucerne meal (20%), desiccated liver, *Torula* yeast (20%) and casein, fish meal or mixtures of amino-acids (10%) or 0·6% of DL-methionine gave a quite significantly protective effect in the prevention of growth retardation in the described experiment. (30 references.) C. V.

Improvement of vitamin levels in canteen kitchen meals. R. Strohecker, jun., G. Wolff and W. Löcher (*Z. LebensmittUntersuch.*, 1960, **112,** 1—10).—A survey of the levels in meals provided by ten factory canteens shows considerable deficiencies [in comparison with D.G.E. (German nutrition board standards)] in provitamin A and vitamins A, B_1, B_2, C and E. Experiments on the addition of a granular prep. of these ingredients to various hot food courses, show that with judicious selection of the food to be fortified, and with simple precautions against undue action of light and air, the vitamins will show 80—100% stability during ~2 hr. in the hot courses. P. S. ARUP.

Statistical procedure for estimating vitamin A assay variation caused by particulate distribution of dry vitamin A in feed samples. R. W. Lehman (*J. Ass. off. agric. Chem. Wash.*, 1960, **43,** 15—20).—The standard deviation of particles of dry vitamin A supplement in a sample of feed would be expected to be $\sqrt{n}$ if the particles were uniform, where n is the average no. of particles in the sample. A size analysis of the particles of various commercial additives show that the particles are not uniform and statistically weighted average particles must be defined. For an approximately S-shaped curve of total no. of particles passing a mesh against the mesh size, the particle diameter below which 60% of the particles are found is a suitable figure. The results lead to rather large assay variations in the normal 40-g. sample. For precise analysis larger samples and higher fortification levels are suggested. E. J. H. BIRCH.

Estimation of the biological potency of vitamin A sources from the maleic values. S. R. Ames and R. W. Lehman (*J. Ass. off. agric. Chem. Wash.*, 1960, **43,** 21—25).—The biological potency of vitamin A prep. may be determined by $SbCl_3$ determinations before and after reaction with maleic anhydride since only those isomers in which the 2—3 and 4—5 double bonds are both *cis*- will react and this excludes the most highly potent all-*trans*- form. The sample is saponified with ethanol–KOH, ether extracted, dried and evaporated and treated with $SbCl_3$ in $CHCl_3$. The blue colour developed is measured spectrophotometrically at 620 mμ. A calibration curve is similarly obtained. A benzene solution of an aliquot from the same residue is shaken with 10% maleic anhydride in benzene and allowed to stand for 16 hr. at 25°, and the vitamin A content determined as before with $SbCl_3$. From these readings the % recovery R can be determined and the maleic value (m) is determined as $100 \times (R - 1{\cdot}1)/88{\cdot}4$. The relative biopotency is given by the empirical regression formula $99{\cdot}5 - 0{\cdot}2m - 0{\cdot}051m^2 + 0{\cdot}000768m^3$, and a table of values is given. A comparison of estimated and observed biopotencies is made. E. J. H. BIRCH.

Use of large samples in the determination of vitamin A in mixed feeds fortified with dry vitamin A supplements. F. H. Tinkler, J. B. Hanley and R. W. Lehman (*J. Ass. off. agric. Chem. Wash.*, 1960, **43,** 25—28).—The particulate nature of vitamin A dry supplements leads to large sampling errors in a 40-g. sample (Lehman, *ibid.*, 1960, **43,** 15). Modifications are described to the standard A.O.A.C. procedure so that a 400-g. sample obtained by riffling a primary sample of at least 25 lb. can be used. The results with samples of cattle, poultry and pig feeds are given. E. J. H. BIRCH.

Method for vitamin A in mixed feeds. D. B. Parrish (*J. Ass. off. agric. Chem. Wash.*, 1960, **43,** 30—34).—The results of vitamin A analysis by a standard method (*ibid.*, 1958, **41,** 57; 1959, **42,** 61) by 14 laboratories on two samples of mixed feed are reported. Carotene results with samples of 20 g., 40 g. and 80 g. were similar. Coeff. of variation for a vitamin A content is 27% for a 40-g. sample containing 670 μg./lb. and 10% for a sample containing 1185 μg./lb. Recommendations for amendment to the standard method are made. E. J. H. BIRCH.

Revision of the animal assay for vitamin D. L. Friedman (*J. Ass. off. agric. Chem. Wash.*, 1960, **43,** 59—62).—Progress in agreeing a procedure for the chemical assay of vitamin D for U.S.P. is reported. It is recommended that the present A.O.A.C. bioassay for vitamin D in milk be extended to all sources (except poultry feeds), and necessary alterations to the procedure are given in detail as amendments to "Official Methods of Analysis" (A.O.A.C.) (5th edn.). E. J. H. BIRCH.

An unidentified water-soluble factor in lucerne which improves utilisation of vitamin A. B. H. Ershoff and H. J. Hernandez (*J. Nutr.*, 1960, **70,** 313—320).—Depletion time was increased by supplements of the indicated fraction and even allowed wt. increase in rats fed on sub-optimal vitamin A (**I**). It was however unable to replace **I** or carotene. No indication of the chemical nature is given. (16 references.) C. V.

Normal growth and development of female chickens without dietary vitamin E or other antioxidants. J. G. Bieri, G. M. Briggs, C. J. Pollard and M. R. S. Fox (*J. Nutr.*, 1960, **70,** 47—52).—Two studies are reported; chicks were fed from hatching with a vitamin-E-free diet containing no added oxidants over a 6—12 month period. Chemical analysis showed that α-tocopherol disappeared from the tissues after five weeks. Compared with the controls, the depleted chicks appeared normal and grew at the same rate, laying eggs at the same time. The various possibilities and implications of these results on nutritional and biochemical functions are discussed. (21 references.) C. V.

Commercial breads as sources of vitamin E for rats determined by the haemolysis test. I. M. Sharman and P. J. Richards (*Brit. J. Nutr.*, 1960, **14,** 85—89).—Weanling rats fed on commercial white bread, reputedly made from ClO_2-treated flour, were given supplements of halibut liver oil to remedy vitamin-A deficiency. This diet gave a regular positive haemolysis test with dialuric acid indicating vitamin-E deficiency. Germ enriched breads and proprietary wholemeal breads gave complete protection. A non-proprietary "brown" bread gave variable results. These findings are in agreement with chemical tests for tocopherol. C. V.

Vitamin E. I. Determination of tocopherols in animal tissues. E. E. Edwin, A. T. Diplock, J. Bunyan and J. Green (*Biochem. J.*, 1960, **75,** 450—456).—The tissues (pig and rat muscle, rat liver and kidney) are ground with solid CO_2–acetone at −70°, and the powder is extracted with boiling acetone. The extract is analysed for tocopherols by a process that includes saponification, removal of sterols, chromatography on floridin earth and two-dimensional paper chromatography (**I**) followed by the Emmerie–Engel colour determination. Separation by **I** is essential because the tissues contain very large amounts of non-tocopherol substances which are included in the Emmerie–Engel colour determination. Factors affecting the

true values of vitamin E determination are discussed. (24 references.) J. N. ASHLEY.

Biological and microbiological methods for detection of the B vitamins. H. Haenel (*Ernährungsforschung*, 1960, **5**, 189—204).—A review with special reference to the limitations of the techniques and the precautions necessary for obtaining reliable results. (35 references.) P. S. ARUP.

Thiamine determination in Italian wheat. M. Filajdić and V. Mikuličić (*Kem. u Industr., Zagreb*, 1960, **9**, 57—58).—Using the thiochrome method values of 0·399–0·547 mg. thiamine per 100 g. wheat grain were obtained for 11 varieties of Italian wheat (1958 crop); the average for all observations was 0·47 mg., with a 12% moisture content. (From English summary.) C. V.

Stability and determination of thiamine in a maize enrichment mixture. M. J. Deutsch, S. S. Schiaffino and H. C. Pillsbury (*J. Ass. off. agric. Chem. Wash.*, 1960, **43**, 45—46).—Application of the A.O.A.C. method for the assay of thiamine in a maize-meal enrichment mixture containing metallic Fe powder and basic substances, leads to difficulties in the extraction procedure and the pH rises after autoclaving. Optimum conditions for the extraction of the thiamine at room temp. with HCl instead of H_2SO_4 are established and the determination completed by the standard method. Analyses carried out by this method after storing the mixture in unstoppered (but covered) flasks for 1 to 12 months showed no alteration in the thiamine content. E. J. H. BIRCH.

Determination of thiamine in enriched cereal and bakery products. L. H. M. Roberts (*J. Ass. off. agric. Chem. Wash.*, 1960, **43**, 47—54).—A detailed comparison by collaborators in the determination of thiamine in cereal products is made for the bread method (*idem, ibid.*, 1958, **41**, 60) and flour method (*Official Methods of Analysis*, 8th edn., A.O.A.C., pp. 819—823) between the use of a direct standard and a procedural standard, and between blanks established by quenching the thiochrome fluorescence in the sample tube with HCl, or by using plain NaOH in the preparation of a separate blank tube. A procedural standard is not necessary except as an occasional check. The two methods of blank determination show no appreciable difference but the HCl method is more rapid. Various changes and alternatives in the official methods are recommended, including substitution of 0·1N-H_2SO_4 for 0·1N-HCl. E. J. H. BIRCH.

Extraction method for thiamine. M. J. Deutsch, S. S. Schiaffino and H. W. Loy (*J. Ass. off. agric. Chem. Wash.*, 1960, **43**, 55—57).—The extraction of thiamine from prep. containing adsorbents by the official methods is found to be unsatisfactory. It is recommended that a 25% solution of KCl in 0·1N-HCl be used for the extraction giving a resulting solution containing $\gtrsim$ 50 mg. of thiamine hydrochloride/ml. which after autoclaving must have a pH $\gtrsim$ 3·5. Oxidation to thiochrome is carried out as in the official method except that the medium is saturated with NaCl or KCl. Results are compared with those obtained by the official method for 25 prep. The presence of absorbents may also prevent utilisation of the vitamin by the body. E. J. H. BIRCH.

Extraction method for adsorbed riboflavin. M. J. Deutsch, H. C. Pillsbury, S. S. Schiaffino and H. W. Loy (*J. Ass. off. agric. Chem. Wash.*, 1960, **43**, 42—43).—Low results for riboflavin by the A.O.A.C. method (*Official Methods of Analysis*, 8th edn.) are obtained when vitamins have been incorporated on an adsorbent. The official method is modified in such cases by substituting a solution of methanol–pyridine–glacial acetic acid (30 : 10 : 10 : 1) for the HCl extraction prescribed. E. J. H. BIRCH.

Micromethod for testing for niacin. E. Medveczky (*Nature, Lond.*, 1960, **186**, 332).—Konno's direct chemical method for differentiating human tubercle bacilli from other *Mycobacteria* by their higher content of nicotinic acid is adapted for use with a smaller wt. of bacteria (1 mg.) by using benzidine instead of aniline for testing for nicotinic acid. Benzidine (3% alcoholic solution) with cyanogen bromide (10% solution) gives a violet-pink colour in presence of nicotinic acid, and the pptg. benzidine crystals are stained. M. D. ANDERSON.

Microbiological assay for total pantothenic acid. E. W. Toepfer (*J. Ass. off. agric. Chem. Wash.*, 1960, **43**, 28—29).—Results of the determination of bound pantothenic acid by the use of prepared pigeon-liver enzyme and intestinal phosphatase (Toepfer *et al.*, *ibid.*, 1954, **37**, 182) show that loss of enzyme activity renders the results unreliable. Attempts from four sources to standardise the pigeon-liver enzyme by the release of pantothenic acid from yeast are reported, but no stable standard by which the enzyme activity can be determined was obtained. E. J. H. BIRCH.

Response of human beings to a low vitamin B_6 diet. K. E. Cheslock and M. T. McCulley (*J. Nutr.*, 1960, **70**, 507—513).—Eight college women were maintained 52 days on a low vitamin B_6 diet. Blood concn. of vitamin B_6 fell to zero in four weeks and remained at this value until 100 mg. supplement of pyridoxine hydrochloride was given. The results indicate that the requirements of this group are >0·5 mg. per day. C. V.

Chromatographic separation of vitamin B_6 components in food extracts. E. W. Toepfer, M. J. MacArthur and J. Lehman (*J. Ass. off. agric. Chem. Wash.*, 1960, **43**, 57—59).—Samples of various foodstuffs after extraction with 0·055N-HCl in an autoclave are chromatographed on ion-exchange columns, and the eluted fractions determined microbiologically with *Saccharomyces carlsbergensis*. Passage through a column of Permutit H-70 at pH 7 leads to retention of the pyridoxamine, which can be eluted with hot 0·5N-HCl. All three components are retained by a Dowex AG 50W-X8 column at pH 4·5 (although a leakage averaging 2% but rising to 8% with large aliquots is observed). Pyridoxal is removed from the column with 80 ml. of hot 0·04M-CH_3CO_2K at pH 5·5, the pyridoxine with 80 ml. of hot 0·05M-CH_3CO_2K at pH 6·1 and the pyridoxamine with 30 ml. of hot 0·2M-K citrate. Pyridoxamine was the most abundant of the B_6 components in yeast, pyridoxal in milk solids and beef, and pyridoxine in groundnut butter. E. J. H. BIRCH.

Modification of the Atkin method for vitamin B_6 assay. N. A. Hurley (*J. Ass. off. agric. Chem. Wash.*, 1960, **43**, 43—45).—The assay of vitamin B_6 by culture of *Saccharomyces carlsbergensis* (ATCC 9080) according to Atkin *et al.*, (*Industr. Engng Chem.*, 1943, **15**, 141) is modified by the substitution of vitamin-free yeast base supplemented with necessary vitamins for the casein hydrolysate medium used by Atkin. The calibration curves afforded by the two media are compared and are little different so that the advantage of the new medium is convenience and uniformity. E. J. H. BIRCH.

Protein complexes. H. B. Klevens (B.P. 818,519—20, 15. 9. 55. U.S., 15.9.54).—[A] Proteinaceous material, e.g., bovine serum albumin, is protected against denaturation by chemicals or heat by treating with a completely or partly fluorinated aliphatic acid, amine, alcohol or alcohol derivative (of $\lesssim$2 *C*), e.g., perfluorocaprylic acid, provided the pH of the mixture is above the isoelectric point of the protein. [B] If the pH is reduced to the isoelectric point, a protein–F-compound complex is precipitated, from which the pure protein can be isolated. F. R. BASFORD.

Ascorbic acid. M. Comar (B.P. 814,574, 1.5.57. Fr., 5.5.56).—Details are given of the extraction of ascorbic acid from leaves of *Yucca guatamalensis* of *Foureroza gigantea* with a two-phase solvent system comprising water, $MgCl_2$ and acetone, and the purification of the product. F. R. BASFORD.

3.—SANITATION

Condensed phosphates, their importance in bottle-cleaning, and problems of disinfection in fully automatic, brushless bottle-cleaning machines. J. Schneider (*Brauwelt*, 1959, **99B**, 1707—1709).—Economical methods of using condensed phosphates, in combination with alkali and silicates, in automatic bottle-cleaning operations are described. Treatment with a condensed phosphate prep. of standardised but limited solubility is recommended at the warm water stage to overcome the effects of hardness. C. L. HINTON.

Stabilising effect of piperonyl butoxide on pyrethrins exposed to ultra-violet light. J. M. Donaldson and J. H. Stevenson (*J. Sci. Fd Agric.*, 1960, **11**, 370—373).—The synergistic effect of piperonyl butoxide on the toxicity of pyrethrins to *Ephestia cautella* is negligible and this insect is therefore particularly suitable for biological assays to find if the synergist has any stabilising effect on pyrethrins during u.v. irradiation. No stabilising effect was demonstrated. E. M. J.

Enriching water with fluorine compounds. M. Groeck (B.P. 819,741, 15.6.56).—The addition of the F compounds to the water is effected in admixture with the re-hardening or hardening reagents. J. M. JACOBS.

4.—APPARATUS AND UNCLASSIFIED

Spectrochemical determination of total strontium in bone, milk and vegetation. R. V. Jury, M. S. W. Webb and R. J. Webb (*Anal. chim. Acta*, 1960, **22**, 145—152).—An effective method for determination of 50—350 p.p.m. of Sr in the ash is described. The spectra are evaluated at 4704·6A. The Ca–Sr fraction in milk and vegetation is precipitated as oxalate before excitation for the spectrographic or flame-photometric determinations described. E. G. CUMMINS.

Journal of Applied Chemistry

The following papers are appearing in the October, 1960, issue

The composition of an atypical gelatin prepared from calf skin
By J. E. Eastoe

Preparation of triallylphosphine oxide
By L. H. Chance and J. D. Guthrie

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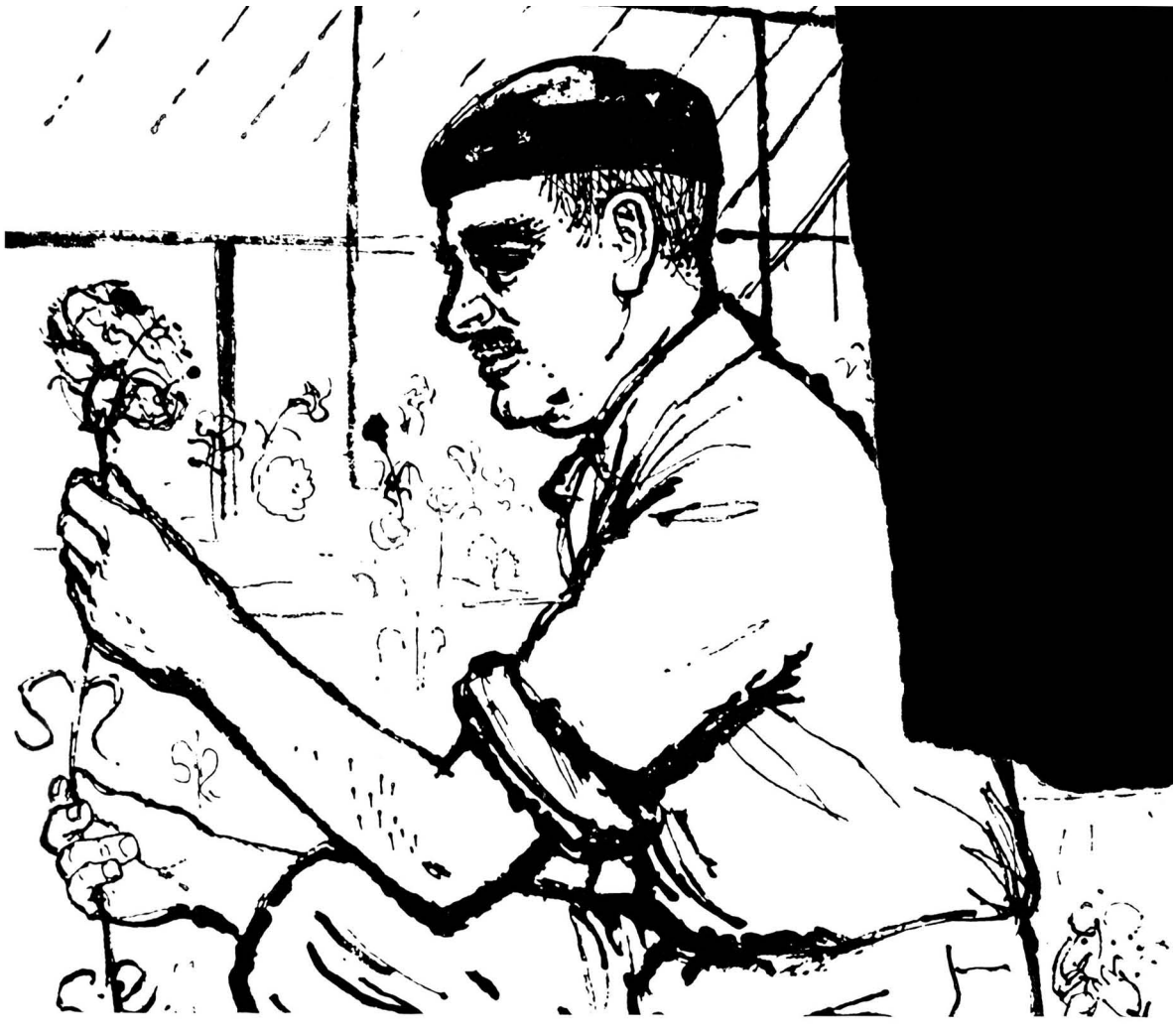

Flowers in the sun

Above the French Riviera the sunlit slopes of the Alpes Maritimes look down on a shimmering sea. Here in the hills the colours are softer, more subdued, the blooms less exotic than the sub-tropical flora of the Côte d'Azur . . . but no less beautiful. Carnations of many kinds (*les Variétés de Nice*) carpet the lower levels and fill huge greenhouses with a profusion of scent and colour. In 1953 however, the beauty and prosperity of this idyllic land was threatened.

Around Cagnes-sur-Mer a virulent enemy of the carnation grower appeared . . . the carnation miner, a prolific pest that musters seven generations between April and November. Early control is essential and, since the grub tunnels inside the carnation leaves and there remains protected from an insecticide deposited on the surface of the foliage, choice of the right product is vital. Phosdrin, the Shell systemic insecticide, has proved to be the one that gives best results ; it is taken up into the plant sap and attacks the carnation miner from *within* the plant and complete control is achieved, usually in little more than a day. It is also effective against other carnation pests such as thrips, red spiders and caterpillars.

For close-to-harvest application on edible crops, Phosdrin is unrivalled, any harmful residues disappearing from the plant within 48 hours. Between them, aldrin, dieldrin, endrin, Phosdrin, D-D and Nemagon can control virtually every world pest.

Phosdrin *Trade Mark*

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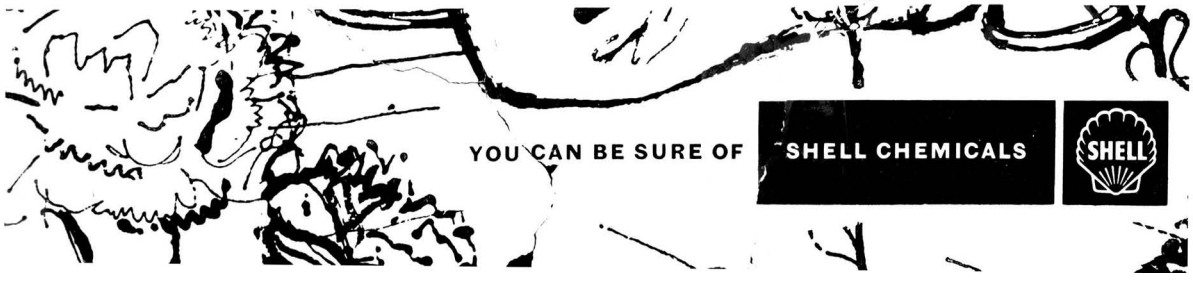

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