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RELATIONSHIP BETWEEN THE EXCHANGEABILITY OF NUTRIENT IONS IN THE SOIL AND ABSORPTION OF PLANTS*

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Introduction

A DISTINGUISHED industrial chemist once stated that many of the present shortcomings in our knowledge of plant nutrition were largely due to Lawes and Gilbert having been too successful. The thought behind this remark was obvious. Before the middle of the last century outstanding practical results were already being achieved by agricultural chemists who analysed soils and plants by relatively simple procedures and thereby deduced how the nutritional requirements of crops could be more adequately met. At that time knowledge of the physiological processes which take place in plants was meagre and, when plant physiology became a subject of active and precise experimentations, the study of nutritional relationships in the field had become so much the province of agricultural chemists that plant physiologists usually turned their attention elsewhere. In subsequent decades there was often surprisingly little contact between those who studied the physiology of nutrition, frequently in fragments of plant tissues grown in artificial media, and the agricultural chemist who grappled with the more immediately practical problems of evaluating the availability of nutrients in the soil and devising how nutritional conditions could be ameliorated.

In this paper it is assumed that emphasis shall be placed on those characteristics of the behaviour of plants which are relevant to determining the relationship between nutrient absorption and the exchangeability of ions in the soil.

Entry of nutrients into plants

Nutrients enter plants as dissociated ions in solution ; the pathways which they follow are similar to those traversed by water, but the mechanism responsible for their movement is different. The driving force behind the transpiration stream is the loss of water from leaves ; mass flow occurs across the root. It is, however, beyond doubt that ions reach the conducting tissues of roots only after they have been conveyed by an ' active ' process, that is to say, one in which energy is utilised.[1] This may sometimes cause the ionic concentrations in the xylem sap to exceed that in the outer medium by a factor of 100 or more.[2] Active transfer depends on energy released by respiration. This has been amply demonstrated by many types of study, for example, investigations of the effects of oxygen tension and the supply of respirable carbohydrates or the action of respiratory inhibitors. The nature of the mechanism, however, still remains unknown.[3] At one time it was widely held that the electron transfer in respiration directly mediated the accumulation of ions, but it is now considered more probable that the connexion between respiration and accumulation is considerably less direct and is possibly linked with protein synthesis.[4] Clearly, however, the process depends on ions being restrained by some product of metabolism in consequence of which they are moved against electrochemical gradients. Whether anions or cations are subject to this process, the other ion moving passively, or whether ions of both signs are transferred, is still uncertain. Here, however, as in many other fields of physiology, increasing appreciation of the value of physico-chemical methods is providing important information. Studies of electrochemical potential differences between the vacuoles or cytoplasm of cells and the external medium has shown that in different parts of the plant kingdom both anions and cations may be subject to active movement.[5]

An appreciable volume of plant roots which lies external to the zone, across which ions can move only by virtue of this active process, is freely accessible to ions by diffusion. This zone

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has been widely referred to as ' free space ' and it has frequently been considered in terms of the Donnan equilibrium. The macromolecules lining the free space are predominantly negatively charged ; thus the system possesses a considerable cation-exchange capacity. Recently, however, it has been suggested that equilibria in the free space are most adequately interpreted in terms of double-layer theory.[6, 7] For present purposes this free space may be regarded rather as an extension of the ion-exchange system in the soil ; it is continuously depleted as active transport conveys ions inwards and simultaneously replenished from the outer medium.

Although in the words of a recent monograph[3] ' In no case in either plants or animals is a relation between active transport and respiration fully understood ', considerable information has been assembled on the factors which influence the rate of passage of different ions through this process. Monovalent ions are, in general, transferred across the root to a considerably greater extent than divalent ones. The latter, however, are likely to be more abundant in superficial exchange phases. In practice the most important variables which affect the absorption of any one ion are likely to be external concentration, the influence of other ions, especially those of the same sign, and the rate at which the ion is utilised for metabolic processes. An increased rate of utilisation of, for example, phosphate or nitrogen in protein synthesis, lowers the internal concentration, thereby accelerating absorption. Thus, the ratios in which ions are absorbed bear a familiar relationship to the growth form of plants. This has sometimes led to the erroneous view that plants actively ' select ' the nutrients they require for growth. It is observed none the less that the absorption process is highly selective in some cases. A well known example is the contrast between plants of maritime origin with other species in the ratio in which sodium and potassium are absorbed.[8]

The extent to which any one ion is absorbed from a constant external supply is influenced by the concentration of other ions in various and complex ways. Competition, comparable to that which occurs in inert ion-exchange systems, occurs in the early phase of uptake. An increase in the concentration of ions of either sign usually depresses the absorption of other ions of that sign, provided the total ionic concentration is sufficiently high. There are, however, exceptions to this rule of which the best known is the effect of calcium ; it has frequently been observed that the addition of calcium may enhance the rate of entry of other cations.[9] The mechanism responsible for this is again unknown, but presumably reflects the effect of calcium on the properties of the cytoplasm. Apart from interactions which directly affect the mechanism of absorption, other types of interaction occur. In some cases increased absorption of one ion may cause the immobilisation of another ; relationships between iron and phosphate have been considered in this regard. Furthermore, considerable interaction occurs in a less direct way. If an increased supply of any one ion accelerates the rate of metabolism of plants, the absorption of all other nutrients used in related processes is likely to be increased. Thus, for example, the provision of nitrogen to plants hitherto but poorly supplied with that element, is likely to increase the absorption of phosphate and others which are used in related metabolic processes.

Although the movement of ions at the root/soil interface is often regarded as unidirectional from the outer medium into the plant, this is in fact not so. Hydrions and carbon dioxide pass outwards from the plant as well as small quantities of numerous organic substances. These latter may provide the substrate for a particularly dense development of micro-organisms, the rhizosphere, in the neighbourhood of the root. Under some circumstances mineral ions also may be released from the plant. This is likely to occur particularly with freely mobile ions, the metabolic requirements for which are greatest in the early stages of the growth. This can occur with potassium in cereal crops. It has been shown that the total potassium content of cereal shoots at harvest may be little more than 50% of that at the time of active tillering[10] and an appreciable part of this loss may occur through roots.

Finally, with regard to the characteristics of the plant which affect absorption, it is important to consider the morphology, distribution and growth of roots in soil. Absorption occurs mainly near the apices of rapidly growing roots and under normal circumstances, or even under conditions of considerable crowding, these tissues will only be in contact with a small fraction of the total volume of soil. The extension of roots, however, brings them progressively in contact with new volumes of soil. Usually we tend to regard the root system of a plant as a single unit, all parts of it functioning in a similar manner. This view, however, lacks experimental

justification; it reflects ignorance of the detailed behaviour of different parts of the system. The possibility that different root zones may absorb ions in contrasting ratios, even though the external supplies are constant, is suggested by the fact that some ions are readily mobile and distributed in plants, while others move unidirectionally only, from roots to aerial tissues. There is some evidence that, as growth progresses, the ratios in which different ions enter may change in contrasting manners in different parts of the root system.

The characteristics of the absorption system of plants which are relevant to a discussion of soil/plant relations may now be briefly summarised as follows:

(1) Ions enter plants by diffusing from the solution phase in the soil into the free space of roots.
(2) Water enters simultaneously with ions but the two processes are controlled by different mechanisms.
(3) The extent to which any one ion is absorbed is influenced not only by its external concentration, but by the concentration of other ions and by metabolic factors.
(4) Hydrions, carbonate and possibly other ions leave roots simultaneously with the absorption of nutrients.
(5) Absorption occurs mainly near the apices of roots. These advance through the soil but are in contact with only a small fraction of its volume at any one time.
(6) The pattern of absorption is not necessarily the same in different parts of the root system and it may change markedly with time.

Clearly no simple chemical test can inform us of the extent to which plants will withdraw nutrients from the soil.

The ionic environment in the soil

In this country what may be called the 'modern' approach to the study of plant/soil nutritional relationships was largely made possible by R. K. Schofield[11] who showed that although, for practical purposes, it might be convenient to regard ions in the soil as in two categories, namely those which were 'available to plants' and those which were not, this distinction has no precise meaning. A thermodynamic approach encouraged clarity of thought especially with respect to ions, such as phosphate and calcium, which enter readily into exchange reactions in the soil. Their behaviour in the two-phase system of 'the labile ionic pool' could be adequately described only in terms of two parameters, the 'potential' or 'intensity' of the system and its 'capacity'. The product of 'potential' and 'capacity' defines the quantity of ions in the system. No general elaboration of the analysis of ionic relationships in these terms is necessary, except to note that from the viewpoint of the present discussion the salient point is that the two different types of measurement of the labile ions in the soil can be made, namely:

(*a*) the *potential* of the system which determines the concentration, or more correctly, the activity of an ion in the soil solution under steady state conditions
and (*b*) the *total quantity* of ions which take part in the equilibria of the labile system. Between different soils this will not be constantly related to the potential because of differences in 'capacity'.

The results obtained by the second procedure are frequently described as measurement of 'exchangeable ions'. However, this term is currently used in more than one way. In soil chemistry 'exchangeable cations' are normally regarded as those which can be displaced from the soil by relatively mild extractants, for example N-ammonium acetate; alternatively the term is now being widely applied to those ions which are isotopically exchangeable. It is here used in the latter sense only.

The exchangeable ions in soils cannot be regarded as a single physico-chemical entity. Many studies of the isotopic exchange of phosphate have shown that whereas part of the phosphate in the soil is rapidly exchangeable with that in the solutions in which it may be suspended, other components exchange more slowly.[12] We must therefore envisage phosphate being restrained on different sites where its free energy is reduced to contrasting extents; suggestions that only two components may be involved would seem an over-optimistic over-simplification.[13]

Calcium appears to behave in a much simpler manner ; the results of many isotopic exchange studies in soils, which do not contain calcium carbonate, have indicated the occurrence of a single first-order reversible reaction.[14, 15] This suggests that all exchangeable calcium is held by the same type of mechanism. However, careful examination has shown the situation to be more complex. A small component of the calcium in soil undergoes isotopic exchange only very slowly, and tracer studies have established that the continued absorption of calcium by plants can cause some ' extra calcium ' which was initially but very sparingly exchangeable to enter the labile pool.[16]

The plant/soil interface

It is now widely realised that, other factors not being limiting, the quantity of ions which will enter a plant root at any instant of time is determined by the concentration or activity of the soil solution, that is to say by the potential of the labile system. Earlier suggestions that ions might enter by ' contact ' exchange directly from solid surfaces are not only unsupported but also incompatible with present knowledge of ionic behaviour on either side of the soil/plant boundary. Ions will enter plant roots only if the absorption mechanism is capable of lowering their free energy below that in the labile soil system.

This instantaneous situation is, however, only of theoretical interest. From the practical viewpoint of agriculture concern must be with the continuing situation, when growing plant roots steadily deplete the soil solution. Absorption will then depend on the extent to which the ionic potential in soil systems is maintained. Two processes must be considered, namely : (1) the dissociation of labile ions from surfaces in the soil ; (2) the diffusion of ions in the solution towards roots.

The need to consider the latter process is obvious when it is remembered that only a small fraction of the total soil volume will be subject to depletion by absorbing roots at any time. However, the maintenance of the soil solution will ultimately depend on the net transfer of exchangeable ions from surfaces in the soil. A question of central interest is the extent to which this depends on the total quantity of exchangeable ions in the system. Over periods which are sufficiently long for the total soil reservoir to be considerably depleted, it would seem reasonable to expect that the extent to which ions enter plants will depend largely on the total quantity which is, or may become, exchangeable.

In the evaluation of soils we are, however, concerned with shorter periods, for example, one or a few growing seasons. It is therefore more relevant to ask questions of the following type : ' If the intensity of a nutrient in two soils is similar but the total quantity in exchangeable form is considerably greater in one, will a crop absorb more ions, other factors being equal ? ' If the answer to this question were always ' yes ', measurements of exchangeable ions would be the appropriate method for evaluating soils. If, however, the answer is ' no ', measurements of exchangeable ions will give valid information on the relative ability of different soils to supply nutrients, only if the ' capacities ' of the labile systems in the soils are similar, as there will then be a constant relationship between the ' intensity ' of the ion and the quantity which is exchangeable.

The consideration of this question is of particular interest because exchangeable ions can be measured relatively easily especially by the use of isotopic tracers. Some experiments which bear on this question with respect to phosphate will now be briefly reviewed.

Relationship between the potential and quantity of exchangeable phosphate in soils and absorption by plants

Two methods which depend on the use of phosphorus-32 have proved of considerable interest, namely, the measurement of isotopically exchangeable phosphate and the Larsen procedure. Isotopically exchangeable phosphate is usually measured by shaking soils in solutions of labelled phosphate, the quantity of exchangeable phosphate (E) in the soil being calculated from changes in specific activity of the solution.[12] Simultaneously the sorption of phosphate (S) by the soil can be measured and, if different soils are examined under constant conditions, the reciprocal of sorption can provide a measure of the relative concentration of

phosphate in the soil solutions, from which differences in the potential can be inferred. The relevant equations are

$$E = yx^t/y^t - x$$
$$S = x - x^t$$

where x and y are the amounts of ^{31}P and ^{32}P originally present in the solution and x^t and y^t are the corresponding values after equilibration for time t.

In the Larsen[17] procedure, labelled nutrient is added to soil in which plants are subsequently grown. The specific activity of the nutrient in plants is subsequently determined and by applying the principle of isotopic dilution, the quantity of nutrients in the soil with which the labelled nutrient was diluted prior to absorption can be calculated. This provides a measure of the quantity of ions in the labile pool (L), that is to say ions which are exchangeable with the soil solution. The relevant equation is

$$L = \frac{y_f(x_p - D)}{y_p} - x_f$$

where x_f and y_f are the quantities of ^{31}P and ^{32}P added to the soil, x_p and y_p the total plant content of ^{31}P and ^{32}P respectively, and D is the phosphate content of the seed. If the quantity of nutrient in the seed is small in relation to the quantity absorbed by the plants, D can be ignored. The description ' A ' value, implying that it was a measure of availability, was introduced for this type of measurement by Fried & Dean[18] who were the first to employ it after Larsen's original study. They, however, regarded the procedure in a somewhat different manner and this has led to misunderstanding. Whereas the procedure as described above depends on incorporating the labelled ions as thoroughly as possible in the soil, Fried & Dean tried to avoid this by applying granular labelled superphosphate. They considered that the results could be interpreted, not in terms of isotopic dilution, but as indicating the availability of the soil phosphate relative to superphosphate which was regarded as a ' standard '. This interpretation does not withstand critical examination. If the labelled fertiliser does not equilibrate with the labile system in the soil before absorption, the relative extent to which the plant will draw on the two sources would be influenced, not merely by the qualities and physico-chemical states of phosphate in them, but also by the contrasting nature of the accompanying ions ; the reasons for this were explained earlier in this discussion. It would appear therefore that the results obtained by this type of procedure are not susceptible to any precise interpretation unless measurements are carried out under conditions which allow isotopic dilution to occur. As will be shown, such measurements are not necessarily related to the ' availability ' of phosphate in plants.

If the same or similar soils are enriched to varying extents with phosphate, relatively small differences usually occur between the E and L values, but they are not always identical. Both characteristically show close linear relationships to the quantity of phosphate absorbed by test plants under standard conditions (Fig. 1). This observation does not, however, establish the direct dependence of absorption on the quantity of isotopically exchangeable phosphate in the soil or on the Larsen value. If the addition of phosphate to the soil did not affect the ' capacity ' of the labile system the relationship shown in Fig. 1 would occur even though absorption depended solely on the potential of the ion in the soil. The validity of this interpretation can be readily demonstrated by comparing widely contrasting soils.

Some years ago, we carried out experiments on the four soils for which details are given in Table I.[12] They ranged from a calcareous alluvium, in which phosphate is regarded as being relatively ' available ' to basaltic soils which show to a marked extent the characteristic of ' fixation '. Extraction of these latter soils with 1% citric acid removed quantities of phosphate comparable with that in the alluvium, but only traces were absorbed by barley plants until fertiliser had been added. The absorption by plants of phosphate from the soils was compared with the reciprocal of sorption, the E value, and the Larsen value. In Fig. 2 the results are shown for an experiment in which three of the soils shown in Table I were enriched with phosphate at different levels and stored either in field capacity or air-dried for 118 days before barley was grown as a test crop for 65 days. Differences in absorption by plants bore some relationship,

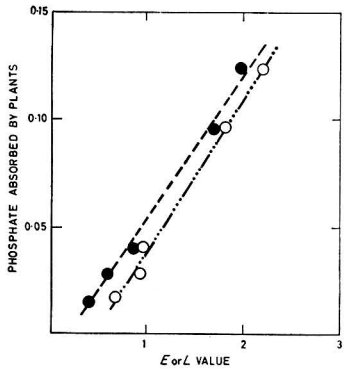

FIG. 1.—*Relationship between absorption of phosphate by barley plants (P) in 43 days, isotopically exchangeable phosphate (E) and the Larsen value (L) in soil 11 examined after storage for 23 days at field capacity (●) and air-dry (○)*

All values expressed as mg. of P/5 g. of soil

Regression equations:
$P = (0{\cdot}74 \pm 0{\cdot}009)\ E - 0{\cdot}034$
$P = (0{\cdot}068 \pm 0{\cdot}009)\ L - 0{\cdot}012$

though not a precise one, to the reciprocal of sorption; they were unrelated to either the E or the L value over the entire series. It would appear therefore that the quantity of isotopically exchangeable phosphate in the soil did not of itself determine absorption over the growth period of these plants; the potential of the ion in the soil system was the determining factor. This implies that measurement of exchangeable phosphate can provide information of practical use only for comparing soils in which the ' capacities ' of the labile systems are similar (see Fig. 1). Accordingly, until exhaustion supervenes, the potential will be determined solely by the extent to which sites on surfaces restrain the ions, that is to say on the ' capacity ' of the labile system.

It was noted earlier that, although the E and L value procedures usually give relatively similar values, they are not necessarily identical. Sometimes marked intraspecific differences in the Larsen value can occur. This was observed particularly in a basaltic soil (Table I, Soil 4) from which plants absorbed very little phosphate until fertiliser was added, and which was of very low phosphate intensity, as indicated by the reciprocal of sorption.[19] In Table II the results obtained when rye and barley were grown in this soil are compared with those obtained on a calcareous alluvium. Whereas on the latter soil the Larsen values were similar for both species, in the basaltic soil the value for rye was twice that for barley. In both soils the Larsen value remained constant between 28 and 56 days. The two crops absorbed closely similar quantities of phosphate from the calcareous alluvium. On the basaltic soil, however, rye absorbed over five times more in the first 28 days, but after 56 days the values for both crops were similar. Presumably a considerable development of roots occurred in barley at a late stage. The larger Larsen value for rye indicated that the specific activity of phosphate absorbed by that crop from the soil was lower. Thus, soil phosphate which had undergone exchange with the added phosphate to only a small extent, and was therefore of low specific activity, had entered rye but not barley. This interspecific difference, which does not occur in soil containing ample supplies of phosphate of relatively high potential, is readily compatible with results of water culture experiments which suggest that rye can absorb phosphate appreciably more readily than barley from very dilute solutions. This observation is of interest in relation to the importance of diffusion processes in determining the extent of absorption to which reference will shortly be made. Beyond this, however, Table II provides further evidence that measurements of exchangeable ions in soil provide no basis for wide generalisations on their phosphate supplying power to plants.

Diffusion of ions in relation to absorption

Water and salts are absorbed from the same zone in the soil by roots, although independent mechanisms are involved. Depending on the ratio in which water and ions enter plants and on the initial composition of the soil solution, the concentration adjacent to the roots may be either enhanced or lowered. The former situation may occur in soils relatively rich in nutrients, but absorption frequently results in the local depletion of the solution phase. Under these latter

Table I

Characteristics of soils investigated

(from Russell *et al.*[12])

Soil	I Calcareous alluvium	II Medium-heavy loam derived from silurian and old red sandstone	III Loam Basaltic	IV Loam Basaltic
Origin	Thames Valley	Edinburgh	Greenhall, Aberdeen	Northern Ireland
' Plant available ' phosphate : extracted in 1% citric acid (mg. P/5 g. of soil)	0·56	0·93	0·89	0·64
Absorption by barley under exhaustion conditions (values relative to soil)	1	0·8	0·2	<0·01

circumstances it is necessary to consider whether the rate at which ions diffuse may become rate-limiting in the overall process of absorption. Suggestions in this direction have been made on the basis of experiments in which the rate of circulation of water in soil had been varied or the diffusion path of ions in soil has been increased by the addition of inert material.[13] The latter evidence, though suggestive, is however subject to other interpretations, since changes in the nature of the rooting medium may influence the development of roots. The contrasting *L* value for barley and rye when grown in phosphate fixing soil (Table II) would, however, be very difficult to explain unless it be assumed that the slow rate of diffusion of phosphate in the soil had limited its entry into rye. If phosphate of relatively high specific activity, such as that absorbed by the barley, had diffused freely through the soil, the considerably less labile phosphate, which showed a very low specific activity, would not have been expected to pass so markedly into solution and thence enter rye. Evidence which implies the importance of diffusion is also available in respect to calcium.[16] Our knowledge of the mechanism of diffusion in the soil is, however, very meagre.

Conclusions

The contrasting extent to which crops absorb ions from the soil throughout their period of growth does not appear to be directly determined by the total quantity of exchangeable ions in the soil. The potential of the ions in the labile system is the most appropriate index of ' availability '. However, widely varying quantities of a nutrient may be absorbed from different

Table II

Absorption of phosphate and Larsen values obtained with barley and rye grown in different soils contained in 500-g. pots

All pots were given a standard fertiliser dressing containing 1·5 g. of P per pot

When results were analysed statistically on a logarithmic basis, the transformed values are shown in brackets

(from Russell *et al.*[19])

Soil No. (see Table I)	Growth period, days	Absorption of phosphate, mg. P/pot			Larsen value, mg. of P/5 g. of soil	
		Barley	Rye	Ratio rye/barley	Barley	Rye
I						
Calcareous alluvian	28	0·175 (2·24)	0·175 (2·24)	1·0	1·82	1·80
	56	0·309 (2·49)	0·316 (2·50)	1·0	1·87	1·86
LSD (P <0·05)		(0·07)			0·08	
IV						
Basaltic	28	0·0057 (0·76)	0·031 (1·49)	5·4	0·94	1·80
	56	0·073 (1·86)	0·058 (1·76)	0·8	0·96	1·86
LSD (P <0·05)		(0·08)			0·16	

soils in which its potential is the same, because of interactions with other nutrients in the absorption process or to the contrasting growth pattern of crops. If, however, the ' capacity ' of the labile ionic system in the two soils is similar, the potential of an ion will bear a constant relationship to the total quantity of exchangeable ions. The measurement of the latter should therefore be a satisfactory basis for comparing the nutrient status of different soils. That this situation occurs widely in practice is indicated by the fact that conventional extraction procedures using, for example, citric acid to extract phosphate, or ammonium acetate to extract calcium, are widely valuable. However, until procedures which directly measure the potential of ions in the soil solution are introduced, difficulties must be expected in intransigent soils. These are illustrated by the lack of relationship between extractable phosphate and absorption, shown by some soils in Table I, and also by Fig. 2. The difficulty of measuring the ionic potential without disturbing equilibria in the soil are obvious. The use of the reciprocal of sorption to provide a basis for comparing qualitatively the potential of different soils is a crude approximation only.

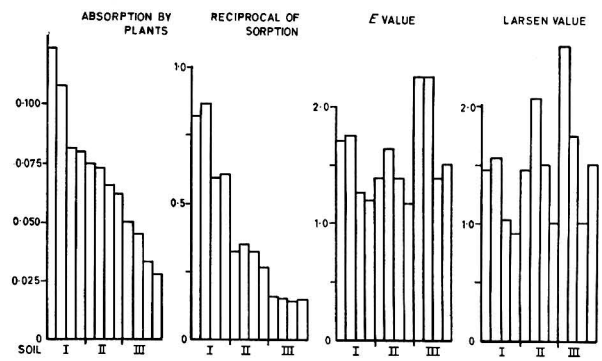

FIG. 2.—*Absorption by rye plants, isotopically exchangeable phosphate* (E), *the Larsen value* (L) *and the reciprocal of sorption for three soils enriched with phosphate to varying levels and stored before use for* 118 *days at field capacity and air-dry*

(Soils described in Table I)
All values expressed as mg. of P/5 g. of soil

The measurement of the concentration of phosphate in solutions of 0·01M-calcium chloride in which soils have been equilibrated has been shown to provide much useful information ; the value of this solution depends on the fact that the ionic balance in the soil is little affected. Recent work[20] suggests that in future it may be possible to make more precise measurements of the ionic potentials in the soil. When this can be done, many of the vague suggestions made here may perhaps be given substance.

Finally, the implications of this situation with regard to the use of tracer procedures deserves comment. Their value as a tool of detailed research on the physical chemistry of the soil has been widely demonstrated ; their importance in studies of plant physiology is equally great. Valuable information has been obtained on many aspects of plant/soil relations, for example, the relative extents to which crops absorb nutrients from different depths. However, despite much optimism and enthusiasm, tracers have as yet helped us little in devising improved methods for the routine testing of the nutrient status of different soils.

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THE COMPONENT FATTY ACIDS OF CHAULMOÖGRA OIL (*TARAKTOGENOS KURZII*, KING)

By A. SEN GUPTA, S. C. MUTHA and A. P. WAGHREY

The fatty acid composition of the seed fat of *Taraktogenos kurzii* has been investigated by carrying out the preliminary resolution of the mixed fatty acids into 13 fractions by low-temperature crystallisation followed by urea adduction and by subsequent identification of the component acids of each fraction by paper chromatography. The presence and the quantity of linoleic acid has been established by ultra-violet spectrophotometry. The composition of the mixed fatty acids as established in the present work consists of chaulmoögric 21·0%; hydnocarpic 19·6%; palmitic 5·8%; myristic 5·9%; gorlic 26·6%; oleic 11·3% and linoleic 4·4%. A gel-like substance of unestablished composition amounting to 5·4%, probably a mixture of partially polymerised/oxidised unsaturated acid was also obtained. The results agree with those of earlier workers for the percentages of chaulmoögric, gorlic, oleic and palmitic acids concerned, but not of hydnocarpic acid. The presence of linoleic and myristic acids in this seed fat has been established for the first time.

Introduction

The fixed oils expressed from the ripe seeds of most of the members of *Hydnocarpus* genus of Flacourtiaceae family are commonly known as chaulmoögra oil, but the true chaulmoögra oil comes from the seeds of *Taraktogenos kurzii*, King, which grows in the dense forests of Burma, Assam and Andaman regions of India, and Chittagong region of East Pakistan.[1]

Chaulmoögra oil and other oils from the closely related species of the family Flacourtiaceae have been used for centuries in oriental countries as an effective remedy of leprosy.[2] Earlier works have shown that the seed fats of various species of Flacourtiacea family are especially characterised by the presence, besides oleic and palmitic acids, of large proportions of chaulmoögric ($C_{18}H_{32}O_2$), hydnocarpic ($C_{16}H_{28}O_2$) and the dienic gorlic acid ($C_{18}H_{30}O_2$) all containing a cyclopentene group.[3–7] The quantitative approach to the fatty acid composition of the seed fats of the Flacourtiaceae was first made by Cole & Cardoso,[8, 9] who separated the liquid acids (gorlic and oleic acids) from the solid acids (chaulmoögric and hydnocarpic acids) by repeated crystallisation at 10° from 80% ethanol. The esters made from the solid acids gave on distillation under high vacuum, a sharp separation of the palmitate and hydnocarpate from the chaulmoögrate, although the first two could not be separated from each other satisfactorily. Similarly distillation of the esters from the liquid fractions did not give such sharp separation. The amounts of the various constituents present in the different fractions were computed on the basis of saponification equivalent, iodine number and optical rotation of the pure acids. The fatty acid composition of the seed fats of *Taraktogenos kurzii*, as established by Cole & Cardoso, was hydnocarpic 34·8%, chaulmoögric 22·5%, gorlic 22·6%, oleic 14·6%, palmitic 4·0%, lower homologues of hydnocarpic acid 0·4% (loss 1·0%). Recently Mehta & Dabhade[10] studied the segregation of the fatty acids of chaulmoögra oil by the urea adduct method and separated five fractions of iodine value varying from 74·4 to 135·2.

A critical examination of the data of Cole & Cardoso raises some questions when considered along with the following general observations[11] on natural fats:

(*a*) Oleic acid is invariably associated with its precursor linoleic acid in all fats in amounts varying from small to comparatively large.
(*b*) In the case of the seed fat saturated acids, while one acid (usually palmitic) predominates, it is invariably accompanied by smaller proportion of the next or lower even-numbered homologues (usually both).

The report of Cole & Cardoso apparently contradicts these facts. While these authors reported the presence of as much as 14·6% of oleic acid, they could not detect even traces of linoleic acid. Similarly the presence of 4% of palmitic acid and complete absence of myristic or stearic acid seem to be rather unusual. In view of the above and also the fact that improved methods are now available for the detection of the fatty acids, it was thought that a reinvestigation of the seed fat of *Taraktogenos kurzii* by utilising some of the modern techniques, may be of use for a better knowledge about the composition of this fat.

Another important consideration, i.e., to find out the influence of the environmental factors on the fatty acid composition, also justified the reinvestigation of the seed fat of this species, since the samples reported by Cole & Cardoso were from the East Indies and no investigation has been reported on the seed fat of *Taraktogenos kurzii* of Indian origin.

The experimental techniques used in the present paper are as follows:

(*a*) low-temperature crystallisation, (*b*) urea-adduct segregation, (*c*) spectrophotometric determination of the linoleic acid, (*d*) qualitative detection of the fatty acids by paper chromatography.

Low-temperature crystallisation was carried out by the method developed by Foreman & Brown.[12] For urea-adduct segregation, the procedure generally advocated by Schlenk[12a] was followed. In the spectrophotometric determination of the unsaturated acids, the method used was that suggested by Hilditch and his co-workers.[13, 14] For paper chromatography the method adopted was essentially that used by Ballance & Crombie.[15]

Experimental

Urea-adduct segregation

The mixed fatty acids were segregated into fractions by stepwise addition of urea to a methanolic solution (1 : 5 w/v) of the acids with filtration after each addition. The proportion of urea to acids was increased gradually starting initially with 1 : 1 ratio. Crystallisation of the adduct was allowed to proceed at each stage for 24 h. at 20°. The various urea adducts obtained were separately warmed on water bath with dilute hydrochloric acid (0·5%) and the liberated acids were extracted with ether. The methanol solution was evaporated and then treated in the same way.

Paper-chromatography

Reverse-phase chromatographic technique was employed in the present investigation, on Whatman No. 3 paper (chromatographic) impregnated with 20–25% of liquid paraffin. For impregnation paper strips (20 × 40 cm.) were immersed in a solution of liquid paraffin (25 ml.) in benzene (75 ml.) for 15 min., drained and lightly blotted and finally dried in air.

The fatty acids in benzene solution (2%) were applied in suitable quantities (total volume 5 μg. for pure acids and 15–20 μg. for mixtures) to the impregnated paper. The chromatogram was developed with 95% equilibrated acetic acid as described by Bernhard *et al.*[16] by the ascending technique[17] for 16 h. The solvent front travelled for 20–25 cm. The developed chromatograms, dried in air, were then kept immersed in cupric acetate solution (10 ml. of saturated solution in 500 ml. distilled water) for 50 min., washed with distilled water and then with running tap water for 1 h. to remove the excess of cupric acetate solution. The copper salts of the fatty acids were detected by immersing the paper in a 2% solution of potassium ferrocyanide for 30 min. followed by washing with distilled water and running tap water for 1 h.

Standard fatty acids

Pure oleic, linoleic, palmitic and myristic acids were obtained from the ' California foundation for Bio-chemical Research ', Los Angeles, U.S.A. Stearic acid was prepared in the laboratory from sodium stearate (E. Merck). Pure chaulmoögric acid was obtained by repeated crystallisation from ethanol at 0° of the fraction of the urea adducts of the mixed fatty acids of chaulmoögra oil rich in that acid. Although pure hydnocarpic and gorlic acids could not be isolated, fractions highly rich in these acids could be obtained by similar technique. All these acids were found to be satisfactory as reference standards for qualitative chromatography.

Results

For the present investigation the seeds of *Taraktogenos kurzii* were procured from the market of Varanasi. The average weight of the whole seed was 2·2 g., the average weight of the kernels was 1·44 g.

Extraction of the seeds with light petroleum (b.p. 60–80°) yielded 33·2% on the weight of the kernels of brownish yellow oil with characteristic odour. Analysis by standard procedures gave the results shown in Table I for the oil and the mixed fatty acids, freed from unsaponifiable portion. The high free fatty acid content of the oil may be due to the fact that the seeds used were from an old stock.

The mixed fatty acids (68·87 g.) were first segregated by the urea-adduct method into seven fractions. Each of the fractions was analysed for iodine value and specific rotation to determine the extent of segregation. The results are shown in Table II.

Although the iodine values for the different fractions indicated a fair degree of segregation, the values for the specific rotation did not give any encouraging indication, as most of the fractions had similar specific rotations. It appeared therefore advisable to separate the mixed fatty acids by low-temperature crystallisation, before the urea treatment, in the hope that separation into three fractions could be effected—one rich in chaulmoögric acid, one in palmitic and hydnocarpic acid and the third rich in oleic, gorlic and linoleic acids. The scheme of crystallisation is shown in Fig. 1.

The initial fraction which separated at 0° from acetone was a gel-like substance, difficultly soluble in hot acetone and light petroleum. This was not further studied. The other fractions A, B, C and D were subjected separately to urea treatment. Both portions A and B yielded two fractions, while portions C and D gave four and five fractions, respectively.

All the thirteen fractions thus obtained were analysed for iodine value, saponification equivalent and specific rotation. The values are given in Table III.

Fractions C_3, C_4, D_4 and D_5 were analysed spectrophotometrically for their linoleic acid content, as it was expected by the separation treatments the linoleic acid had concentrated in these fractions only. The results are tabulated in Table IV. The percentage of linoleic acid in the mixed fatty acids calculated from those of the fractions of C_4, D_4 and D_5 is 4·3%.

All thirteen fractions were then subjected to paper-chromatographic analysis for the identification of their constituent fatty acids. As reference standards the R_F values of the different pure acids were determined first under the experimental conditions. Since R_F values require exact maintenance of the experimental conditions for reproducibility, it was found more convenient to express the movement of an acid with reference to chaulmoögric acid and the term R_M was used to indicate this. The values of R_F and R_M of the pure fatty acids are given in Table V.

The conclusions about the possible component fatty acids of the different mixtures were based on the iodine values, saponification equivalents and R_M values. The results of the paper-chromatographic analysis are shown in Table III.

When the constituents of the different fractions were known qualitatively from the chromatographic study, the percentage compositions were calculated from the saponification equivalents and iodine values. The specific rotation results were not reliable for quantitative purposes. It is evident from Table III that none of the fractions except C_4 contains more than three fatty acids. So by solving three algebraic equations, one based on iodine values, the second based on saponification equivalents, and the third based on the total percentage of the three constituents,

Table I

Characteristics of chaulmoögra oil and mixed fatty acids (free from unsaponifiable matter)

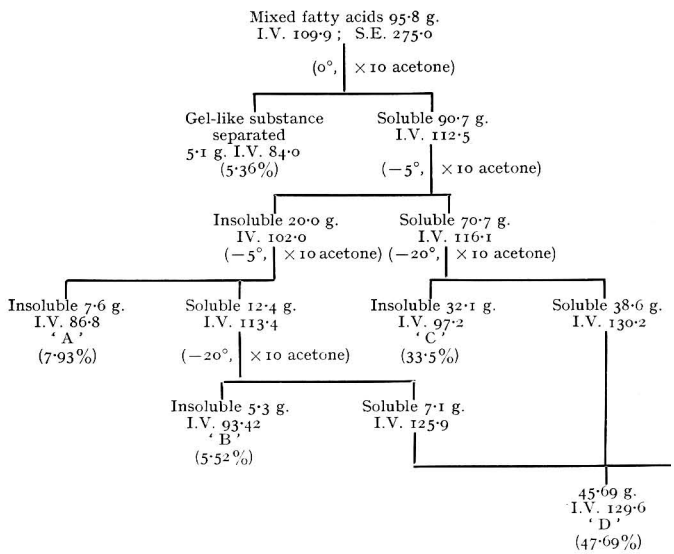
	Oil	Mixed fatty acids
Saponification equivalent	287·7	275·0
Iodine value (Wij, 30 min.)	105·2	109·9
Specific optical rotation $[\alpha]_D^{30}$	+41·0	+42·2
Refractive index at 40°	1·5447	—
Unsaponifiable (% by wt.)	0·29	—
Free fatty acid (% oleic)	13·9	—
$E_{1cm.}^{1\%}$ at 234 mμ isomerised at 180° for 60 min.	—	33·90
$E_{1cm.}^{1\%}$ at 234 mμ unisomerised	—	2·1 (neglected)
% of linoleic acid	—	3·74

Table II

Analysis of fractions obtained by the urea-adduct method

Fraction	Weight, g.	% of total weight	Iodine value (Wij, 30 min.)	Specific rotation $[\alpha]_D^{30}$
A	7·40	10·76	65·8	+28·8
B	13·38	19·47	82·9	+42·0
C	10·16	14·79	96·4	+48·4
D	13·45	19·56	102·5	+43·6
E	3·53	5·14	110·1	+44·1
F	9·10	13·04	122·5	+33·8
G	11·85	17·24	164·9	+48·2

The computed values for mixed fatty acids from these results are iodine value 107·7; sp. rotation $[\alpha]_D$ at 30° + 41·8



(I.V. = iodine value; S.E. = sap. equiv.; × 10 acetone means with 10 vol. of acetone)

FIG. 1.—*Low-temperature crystallisation of mixed fatty acids after removal of unsaponifiable matter*

Table III

Characteristics and results of the paper chromatographic study of the fractions obtained by low-temperature crystallisation and urea-adduct formation

Fraction	Specific rotation, $[\alpha]_D^{30}$	Iodine value	Saponification equivalent	No. of spots observed	R_M value	Possible components
A_1	+52·36	87·6	277·1	2	0·99 1·50	Chaulmoögric, palmitic Hydnocarpic
A_2	+42·30	82·2	272·7	2	0·99 1·50	Chaulmoögric, palmitic Hydnocarpic
B_1	+48·27	82·6	255·2	2	0·94 1·34	Palmitic Hydnocarpic and gorlic
B_2	+45·80	101·4	265·0	2	0·96 1·33	Palmitic Hydnocarpic and gorlic
C_1	+34·50	64·4	268·6	2	0·97 1·50	Palmitic, chaulmoögric, Hydnocarpic
C_2	+45·17	81·0	274·5	2	0·99 1·50	Palmitic, chaulmoögric Hydnocarpic
C_3	+64·00	98·4	260·1	2	1·00 1·50	Chaulmoögric Hydnocarpic
C_4	+63·50	118·6	270·8	3†	1·00 1·30 1·50	Chaulmoögric Linoleic and gorlic Hydnocarpic
D_1	+18·16	75·1	271·0	2	0·92 1·52	Oleic and palmitic Hydnocarpic
D_2	*	93·0	273·4	2	0·91 1·48	Oleic Hydnocarpic
D_3	+40·78	111·50	266·5	2	0·91 1·27	Oleic Gorlic and myristic
D_4	*	157·10	270·4	1	1·30	Gorlic, myristic and linoleic
D_5	+42·07	164·20	274·6	2†	1·1 1·32	Gorlic Linoleic and myristic
Gel	—	84·0	394·4	—	—	—

Computed values for total mixed fatty acids :

Iodine value	109·6
Sap. equiv.	276·3
Sap. equiv. (without gel)	271·3

* Specific rotation could not be determined as there was insufficient material
† Two spots diffused into one another

the percentage compositions of the fatty acids of the different fractions were calculated. In the case of fraction C_4, since it contains linoleic and gorlic acids, whose iodine values and saponification equivalents are nearly the same, they were assumed to be behaving as one single component. When the total percentage of gorlic and linoleic acids was obtained, the percentage of linoleic acid, as determined spectrophotometrically, was deducted from the total of the two to get the value for gorlic acid.

The percentage compositions as calculated from these equations are given in Tables VI and VII.

Table IV

Spectrophotometric analysis of fractions C_3, C_4, D_4 and D_5

Fraction	% of total	Iodine value	$E_{1cm.}^{1\%}$ at 234 mμ, isomerised at 180° for 60 min.	Linoleic acid, %
C_3	10·84	98·4	Negligible	Negligible
C_4	11·85	118·6	41·26	4·6
D_4	2·42	157·1	96·70	10·7
D_5	18·61	164·2	169·10	18·7

Table V

R_F and R_M values of the pure acids

Acids	R_F	R_M	Acids	R_F	R_M
Stearic	0·17	0·59	Linoleic	0·38	1·30
Palmitic	0·28	0·95	Chaulmoögric	0·29	1·00
Myristic	0·41	1·40	Hydnocarpic	0·45	1·50
Oleic	0·27	0·91	Gorlic	0·32	1·10

Table VI

Distribution of acids in different fractions on a percentage basis

Fraction	Chaulmoögric	Hydnocarpic	Palmitic	Myristic	Gorlic	Oleic	Linoleic
A_1	88·4	7·7	3·9	—	—	—	—
A_2	71·7	17·5	10·8	—	—	—	—
B_1	—	67·4	24·5	—	8·1	—	—
B_2	—	21·4	34·8	—	43·8	—	—
C_1	54·1	15·2	30·7	—	—	—	—
C_2	77·8	9·5	12·7	—	—	—	—
C_3	23·0	77·0	—	—	—	—	—
C_4	41·2	32·4	—	—	21·8	—	4·6
D_1	—	21·4	19·0	—	—	59·6	—
D_2	—	28·7	—	—	—	71·3	—
D_3	—	—	—	25·2	48·8	26·0	—
D_4	—	—	—	13·5	75·8	—	10·7
D_5	—	—	—	9·6	71·7	—	18·7

Discussion

For comparison, the findings of the earlier workers on the compositions of the seed fat of the Flacourtiaceae family are tabulated in Table VIII with those obtained in the present investigation.

It is evident that there is some similarity between the results of the present investigation and those of Cole & Cardoso,[9] so far as the percentage of chaulmoögric, gorlic, oleic and palmitic acids are concerned, but for hydnocarpic acid there is a striking difference. While Cole & Cardoso reported 34·9% of hydnocarpic acid, the present investigation indicates approximately half of that amount (19·6%).

Table VII

Composition of mixed fatty acids

Fraction	% of total mixed fatty acids	Chaul-moögric	Hydno-carpic	Palmitic	Myristic	Gorlic	Oleic	Linoleic
A_1	5·7	5·1	0·4	0·2	—	—	—	—
A_2	2·2	1·6	0·4	0·2	—	—	—	—
B_1	2·8	—	1·9	0·7	—	0·2	—	—
B_2	2·7	—	0·6	0·9	—	1·2	—	—
C_1	6·5	3·5	1·0	2·0	—	—	—	—
C_2	4·4	3·4	0·4	0·6	—	—	—	—
C_3	10·8	2·5	8·3	—	—	—	—	—
C_4	11·9	4·9	3·8	—	—	2·6	—	0·6
D_1	6·4	—	1·4	1·2	—	—	3·8	—
D_2	4·9	—	1·4	—	—	—	3·5	—
D_3	15·3	—	—	—	3·8	7·5	4·0	—
D_4	2·4	—	—	—	0·3	1·8	—	0·3
D_5	18·6	—	—	—	1·8	13·3	—	3·5
Gel	5·4							
Total	100·0							
	Gel							
Total % by wt.	5·4	21·0	19·6	5·8	5·9	26·6	11·3	4·4
Total mole-%	3·7	20·4	21·3	6·2	7·1	26·1	11·0	4·2

Computed values for mixed fatty acids :

Iodine value	110·0
Sap. equiv.	276·2
Sap. equiv. (without gel)	269·7

Table VIII

Composition of seed fat of different species of Flacourtiaceae family

Species	% weight of acid							
	Hydnocarpic	Chaulmoögric	Gorlic	Oleic	Linoleic	Palmitic	Myristic	Lower homologues
Taraktogenos kurzii[9]	35·3	22·7	22·8	14·8	—	4·0	—	0·4
Hydnocarpus weightiana[18]	48·9	27·1	12·3	6·5	—	1·8	—	3·4
Hydnocarpus anthelmintica[9]	69·3	8·9	1·4	12·6	—	7·7	—	0·1
Carpotroche brasiliensis[8]	46·1	24·9	15·8	6·4	—	6·8	—	—
Oncoba echinata[8]	—	75·2	14·8	2·2	—	7·8	—	—
Calancoba welwitschii[19]	—	75·0	17·4	0·8	—	6·8	—	—
Hydnocarpus weightiana[20]	46·4	21·5	10·0	12·5	—	9·6*	—	—
Taraktogenos kurzii (present work)	19·6	21·0	26·6	11·3	4·4	5·8	5·9	5·4 (gel)

* includes lower homologues of chaulmoögric acid

Moreover the present investigation definitely establishes the presence of 4·4% of linoleic acid in the mixed fatty acids, while Cole & Cardoso do not report the presence of linoleic acid. Of the saturated fatty acids, Cole & Cardoso reported about only 4% palmitic acid, but in the present investigation the presence of 5·9% myristic acid in addition to 5·8% palmitic acid has been indicated.

When the mixed fatty acids were subjected to low-temperature crystallisation, a gel-like substance (sap. equiv. 394·0; iodine value 84·0) was obtained which amounted to 5·4% of the total. This fraction, difficultly soluble in hot acetone, light petroleum and benzene, was probably a mixture of partially polymerised/oxidised unsaturated acids.

It is evident that the techniques used in this investigation gave a very good segregation and qualitative detection of the constituent fatty acids. The divergence of the results obtained from those of Cole & Cardoso[9] is considered to be due mainly to the better analytical methods available, but the different geographical source of the seeds may have been a contributory cause.

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TANNINS AND POLYPHENOLS IN CAROB PODS (*CERATONIA SILIQUA*)*

By EDNA NACHTOMI and EUGENIA ALUMOT

Separation of condensed tannins from sugars and low-molecular weight polyphenols was achieved by cold-water extraction of carob pod meal. The condensed tannins were isolated by subsequent hot-water extraction of the meal and analysed by permanganate titration, alkaline hydrolysis and fusion, paper chromatography, reactions for the presence of *leuco*-anthocyanins and catechins. They were not soluble in commonly used tannin solvents. Although the presence of *leuco*-anthocyanins and flavanols was shown by general reactions, no monomeric substances of this group were detected in ripe carobs. Extracts from green carobs contained several catechins and *leuco*-anthocyanins, that may be regarded as possible precursors of condensed tannins.

Gallic acid was the main constituent of the low-molecular weight fraction obtained from ripe carobs and which was soluble in organic solvents.

Introduction

Carobs have been reported to depress the growth of chicks,[1, 2] but the cause for this effect is not known. Alumot & Nachtomi[3] found appreciable amounts of tannin-like compounds and other polyphenols in carobs. Further characterisation of these compounds as possible growth depressing factors is clearly of interest.

Although methods for extraction, separation and identification of plant polyphenols are known,[4, 5] the properties of high-molecular condensed tannins of many plants remain obscure. The low solubility of carob tannins in organic solvents, their occurrence as complex mixtures and the high concentration of sugars in carobs complicate the task of separation and identification.

Experimental

Materials and methods

The material used throughout this work was prepared from locally grown carob pods (Cyprus variety) by removing the seeds and grinding in a hammer mill fitted with a 2-mm. sieve.

Direct hot-water extracts served for the general characterisation of the polyphenols. Permanganate titration was used to determine the reducing polyphenols before and after precipitation with gelatin.[6] Flavanols and *leuco*-anthocyanins were determined by the method of Swain & Hillis.[7]

Cold water extraction.—Sugars and low-molecular weight polyphenols were removed from carobs by stirring twice with 8 vol. of distilled water and filtering. The extraction was repeated twice with 5 vol. of water. The aqueous extracts were concentrated under reduced pressure in nitrogen-atmosphere, and the syrup produced was extracted several times by stirring with ethyl acetate. After removal of the solvent by distillation, the extracted material containing the polyphenols was finally dried in a desiccator.

Hot-water extraction.—The residue remaining after extraction with cold water was extracted four times with hot water, with volumes similar to those described above. The material was refluxed for 2 h. each time and the solution filtered hot. The combined extracts were concentrated under reduced pressure in a current of nitrogen.

The concentrated extract was shaken several times with ethyl acetate and the solvent layer analysed after removal of the solvent by distillation. Ethyl acetate-insoluble material was purified by precipitation with ethanol, the precipitate washed with small quantities of ethanol and ethyl acetate and dried in vacuum. The light-brown amorphous residue was analysed for reducing substances by the permanganate method, for the presence of *leuco*-anthocyanins and compounds containing a phloroglucinol nucleus by spectrophotometry[7] and for ether-soluble degradation products, obtained after alkaline hydrolysis and fusion, by paper chromatography.

Alkaline hydrolysis.—Fifty mg. of the above powder were dissolved in 20 ml. of 12% (w/v) potassium hydroxide in 50% (v/v) ethanol and refluxed for 1 h. The solution was cooled,

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acidified with a few drops of 5N-sulphuric acid and the excess acid neutralised with 10% (w/v) sodium carbonate. The neutral solution was extracted three times with ether and the extracts evaporated to dryness on a water bath.

Alkaline fusion was carried out according to the method of Roux.[5]

Chromatography.—Two-dimensional descending chromatography[8] was employed, using Whatman No. 1 (18 × 22½ in.) filter paper. The chromatograms were developed successively with n-butanol/acetic acid/water (4 : 1 : 2·2 by vol.) overnight and with 2% (v/v) aqueous acetic acid during 6 h. in the second direction. Spots were located under ultra-violet light and by spraying with colour-developing reagents: ammoniacal silver nitrate,[9] ferric chloride–potassium ferricyanide and vanillin–hydrochloric acid.[10] Identification of the spots was effected by co-chromatography with reference substances and reactions with specific reagents.

In order to follow the formation of tannins in the fruit, green carobs (8 weeks before ripening) were also examined for polyphenols.

Results

The reducing power of gelatin-precipitated polyphenols, determined by the permanganate method, was 150–200 (expressed as mequiv. or m.-atoms of hydrogen) per 100 g. of carobs. Small titration values were obtained after gelatin precipitation, indicating that most of the polyphenols were precipitated by gelatin.

When polyphenols were determined in green carobs, half of the reducing groups remained in solution after gelatin precipitation. This may be due to the higher degree of tannin condensation in ripe carobs as compared with the green ones.

A portion of the carob solution was heated with equal volume of 2N-hydrochloric acid for 20 min. to test for *leuco*-anthocyanins.[11] A brown-red colour was obtained which turned crimson when extracted into isoamyl alcohol; therefore *leuco*-anthocyanins were suspected to be present initially. Further confirmation was provided by the method of Swain & Hillis.[7] A typical spectral curve was obtained, which showed a maximum at 550 mμ. The presence of flavanols was also indicated by application of the above mentioned method of Swain & Hillis.

The cold-water extract

This constituted about 50% by weight of the carobs and contained mainly sugars (sucrose, glucose, fructose and xylose). Permanganate titration gave a small value, which did not change after gelatin precipitation. The polyphenols extracted by ethyl acetate, when examined by two-dimensional paper chromatography, revealed one large spot and traces of several other spots giving typical phenol reactions. No reaction occurred within vanillin–hydrochloric acid. The large spot was shown to be due to gallic acid; it gave a grey colour with ferric chloride–potassium ferricyanide and ammoniacal silver nitrate; the addition of an authentic specimen resulted in the intensification of the spot. Further confirmation was obtained by paper chromatography with aqueous saturated phenol as a developing solvent. Again the R_F value was identical with that of gallic acid. The smaller spots were not identified. They fluoresced under ultra-violet light.

Hot-water extract

The hot-water extract constituted about 8% by weight of the carobs. Results of chromatography of the ethyl acetate-soluble compounds from hot-water extract are presented in Fig. 1. Again in this case no colour reaction developed with vanillin–hydrochloric acid. The spots were located by ferric chloride–potassium ferricyanide, ammoniacal silver nitrate and by ultra-violet irradiation. The colours obtained are given in Table I.

The large spot (No. 10 in Fig. 1) was identified as gallic acid. Spots Nos. 11–13 were green to yellow before development. Spots Nos. 1–7 may be due to planar flavanoid compound (low R_F value in aqueous solvents[12]).

As in this study attention was paid mainly to the compounds insoluble in ethyl acetate and which form the bulk of polyphenols in carobs, no further attempt was made to identify the trace substances found in water extracts.

The substances insoluble in ethyl acetate, purified by washing with alcohol and ethyl

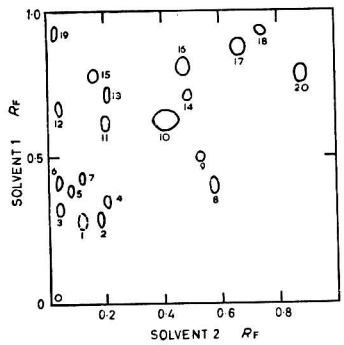

FIG. 1.—*Two-dimensional chromatogram showing the polyphenols from hot water extract soluble in ethyl acetate*
Solvent (1): n-butanol/acetic acid/water (4 : 1 : 2·2)
Solvent (2): 2% aqueous acetic acid

acetate, were slightly soluble in acetone. They did not move on paper in the two solvents used, namely n-butanol/acetic acid/water and aqueous acetic acid. They gave an intense colour reaction with vanillin–hydrochloric acid on paper and by the Swain & Hillis test, indicating a phloroglucinol unit in their structure.

The ether extract after alkali fusion revealed several spots with the ferric chloride–potassium ferricyanide reagent, indicating degradation products (Fig. 2). Spots Nos. 3, 4, 7 and 11 also gave colour reaction with vanillin–hydrochloric acid. Spots Nos. 2, 3, 4 and 7 were identified as gallic acid, phloroglucinol, pyrogallol, and resorcinol, respectively (pyrogallol may be formed from gallic acid by decarboxylation).

The ether extract of the alkaline hydrolysate contained three substances. Gallic acid and phloroglucinol were identified. The third compound is suspected to be an anthocyanin: it was pink on the chromatogram before development with colour reagents. Its R_F values were similar to those of Spot No. 1 in Fig. 2. A dark-red precipitate, formed during alkaline hydrolysis, was not extractable by ether.

It is clear from the above results that the main constituents of carob polyphenols are condensed tannins containing the flavan nucleus and insoluble in the usual organic solvents. As no potential precursors of these compounds were found in the ripe carobs, examination of green carobs was carried out from this point of view.

Extraction of green carobs

Green carobs were extracted directly with hot water under reflux, as they contain only traces of sugars. A cream-coloured turbid solution was obtained, which gave a white-yellowish powder when dried. The ethyl acetate-extractable products were submitted to paper chromatography (Fig. 3).

All the lettered spots gave the characteristic colouration with vanillin–hydrochloric acid. Numbered spots gave only reactions with the general phenol reagents. Spots Nos. 1–3 gave a violet and Spot No. 5 a blue fluorescence under ultra-violet light. From the R_F values, colour reactions and comparison with authentic substances, spots A, B and D were identified as

Table I

Colour reactions of trace substances, soluble in ethyl acetate (ripe carobs)

Substance (Spot No. in Fig. 1)	Fluorescence ultra-violet	Ferric chloride–ferricyanide	Ammoniacal silver nitrate	Vanillin–hydrochloric acid
1–7	—	blue	brown	—
8–10, 14–17	—	blue	grey	—
11–13	violet	yellow	brown	—
19	blue	blue	brown	—
18, 20	—	grey brown	grey	—

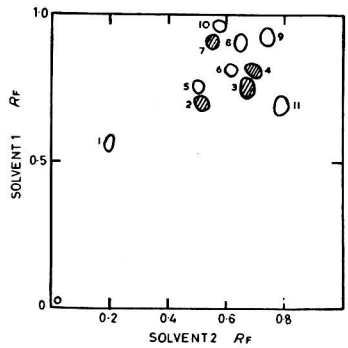

FIG. 2.—*Two-dimensional chromatogram showing the polyphenols extracted by ether after alkali fusion*
Solvents as in Fig. 1
(The more intense spots are shaded)

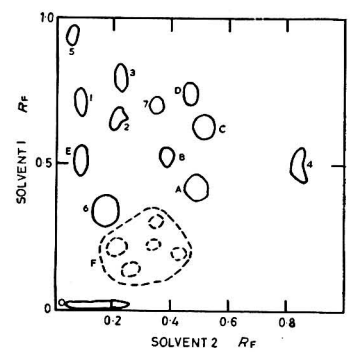

FIG. 3.—*Two-dimensional chromatogram of green carobs polyphenols*
Solvents as in Fig. 1

(+)-gallocatechin, (−)-epicatechin and (−)-epicatechin gallate, respectively. Spot C contained a mixture of (+)-catechin and gallic acid. The group of spots designated F was eluted and identified as *leuco*-anthocyanins by the method of Roberts *et al.*[13] Because of the presence of asymmetric carbon atoms, many isomeric substances are possible in this group.

Discussion

The first indication as to the complexity of carob tannins is their lack of solubility in typical tannin solvents such as ethyl acetate, methanol and ethanol, so that these substances cannot be examined by paper chromatography without previous degradation. The general reactions of these compounds and the products formed from them by alkaline hydrolysis and fusion are typical for condensed tannins, i.e., polymerisation products of flavan units (Freudenberg[14]).

Catechins and *leuco*-anthocyanins found in the green carobs may be regarded as possible precursors of those condensed tannins. Because of the high degree of polymerisation, no free monomers are found in ripe carobs. *Leuco*-anthocyanins were found in the leaves of the carob tree by Bate-Smith & Lerner.[15] Gallic acid, found in greater amounts in ripe than in green carobs, may be formed as a by-product of enzymic oxidation of flavanols.[16]

The possible precipitating action of polyphenols on proteins and enzymes may explain the disturbances caused by carobs to animals, but this problem needs to be clarified in further work.

Acknowledgments

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ASEPTIC AUTOLYSIS IN RABBIT AND BOVINE MUSCLE DURING STORAGE AT 37°

By J. G. SHARP

The changes in a number of protein fractions in rabbit and bovine muscle have been observed during aseptic storage at 37°. Continuous breakdown of protein took place with formation of TCA-soluble non-protein N, the rates being 20·7 μmoles and 10·3 μmoles N/g./day for rabbit and beef respectively. No change was detected in the solubility of the collagen fraction over 6 months' storage. The fine structure of the myofibrils remained apparently unchanged during this period and the main autolytic effect of the cathepsins present would appear to be concentrated on the sarcoplasmic proteins. The degree of disintegration of muscle structure caused by homogenisation under standard conditions was much greater in muscle held for 30 days at 5° than in muscle after the same period at 37°.

Introduction

The course of the physical changes which take place in the texture of meat after the death of the animal has been well established for many years. The meat becomes tougher with the development of *rigor mortis* and then, in the *post rigor* period, it becomes continuously less tough, reaching an acceptable degree of tenderness after 10 to 18 days at 0° to 5°.

Despite many physical, biochemical and histological studies however, the main causes of this change in texture *post rigor* are still undefined. A comprehensive review of the literature in this field has recently been made by Whitaker.[1] Zender and his colleagues,[2, 3] in their recent studies of aseptic autolysis, observed almost complete breakdown of texture in rabbit and lamb muscles during storage at 25° and 37°. Locker, using N-terminal group analysis, found little evidence of proteolysis in bovine muscle during 14 days at 2° and 3 days at 21°.[4] On the other hand, Russian workers[5] by using a modified technique have shown a large increase in free N-terminal groups of a myosin fraction extracted from meat after 6 days at 10°. Kronman & Winterbottom[6] have followed by electrophoresis and ultracentrifugation the changes occurring in the water-soluble proteins of bovine muscle during 7 days at 3°.

The autolytic changes are believed to be due to the action of the proteolytic enzymes, the group of ' cathepsins ', known to be present in relatively low concentrations in muscle tissue. With the application of newer methods of fractionation, these cathepsins are beginning to attain a certain degree of definition with regard to the number present and to their individual specific proteolytic activity. Thus Sliwinski *et al.*[7] have separated from bovine muscle three proteolytic enzyme fractions with optimum activities at pH 4–5, 8–9 and 10, respectively, and

Koszalka & Miller[8] have proved the presence of a proteolytic enzyme in rat muscle with optimum activity at pH 8·5–9·0. Dvorak followed the changes in the proteolytic activity of bovine muscle during storage at 5° by the activity of aqueous extracts on synthetic substrates.[9] The activity of the extracts increased with time of storage of the muscle at 5°, indicating a release of enzymes during autolysis.

The aim of the present study was to obtain quantitative data on the autolytic changes which take place in certain protein fractions of muscle during aseptic storage at 37°, particular attention being given to the main connective tissue protein collagen. A brief note on the work has already been published.[10]

Experimental

Preparation of sterile samples

Rabbit muscle (*Method* 1).—In the first series of tests the *longissimus dorsi* muscles of rabbits were dissected within 10 min. *post mortem*, under conditions in which bacterial contamination was reduced to a minimum. To kill any incidental surface contamination, the complete muscles were submerged in 70% ethanol and cut transversely into sections about 2–3 cm. long. Immediately after being cut, each section was removed from the bath and ignited, then again dipped momentarily in ethanol and ignited. The individual sections were sealed in sterile jars and held at the specified storage temperature. The flamed sections had a denatured pellicle about 1–2 mm. thick, but the interior tissue showed no signs of heat treatment in either colour or texture. Thermocouple observations indicated that the temperature of the interior tissue did not rise above 37°. After storage the denatured pellicle was removed from the sections and samples of the interior tissue were taken for analysis.

Rabbit muscle (*Method* 2).—Attempts were made to obtain sterile samples without using the alcohol flaming technique. In one test (Rabbit A, Table I), the muscles were dissected and sectioned under carefully controlled aseptic conditions, but only 30% of the samples proved sterile after storage.

In a second test (Rabbit B, Table I), the surfaces of the exposed *longissimus dorsi* muscles were sterilised by painting with an alcoholic solution of the dyes, Crystal Violet and Brilliant Green. Samples from the interior of the muscles were then removed aseptically. At least 50% of the samples obtained by this simpler procedure were found to be sterile after storage at 37° and, with modifications, the procedure was used successfully for obtaining sterile samples of bovine muscle.

Bovine muscle.—Complete sections of the loin containing the last four ribs were taken from carcasses which had been held for 24 h. *post mortem* at 0–5°. The whole surface of the section was flamed with ethanol and the superficial muscles and fat layers excised aseptically to expose the *longissimus dorsi*. The surface of the muscle was sterilised by painting with the above dye solution and the sheath (epimysium) and underlying tissue were removed aseptically to a depth of about 1 cm. to expose the main bulk of the muscle. Samples weighing 6–9 g. were removed aseptically and sealed in sterile containers either in air or in nitrogen for subsequent storage. This procedure was relatively simple to operate and was successful in providing a large number of sterile samples from the same muscle. About 70% of the samples remained sterile after storage at 37°.

Adrenaline treatment of rabbits

Samples of *longissimus dorsi* of high ultimate pH, 6·4–6·8, were obtained by method 1, from rabbits injected subcutaneously with 1·5 mg. of adrenaline 4 h. *ante mortem*.

Analytical methods

Extraction of protein fractions.—In the preliminary tests the extraction procedure was based on the procedure for extraction of ' soluble ' collagen, namely extraction at pH 8·9 with 0·2M-disodium phosphate buffer followed by extraction with 0·1M-citrate buffer at pH 3·8.[11, 12] In later tests, the muscle samples were homogenised and extracted firstly with 0·1M-potassium chloride, secondly with disodium phosphate buffer and finally with citrate buffer.

As the work progressed, modifications, which will be evident from Tables I and II, were made in the extraction procedure. It soon became clear, for example, that any 'soluble' hydroxyproline (OHP) present was extracted in 0·1M-potassium chloride and consequently, in later tests, the phosphate and citrate extractions were omitted.

The complete procedure when applied to muscle immediately *post rigor* gave a rough fractionation into sarcoplasmic proteins soluble in 0·1M-potassium chloride, myofibrillar proteins soluble in 0·2M-disodium phosphate, a small residual fraction soluble in 0·2M-citrate, and a large residue of connective tissue and denatured proteins. Any fraction similar to the 'soluble' collagen of Orekhovitch[11] should be present in the phosphate and citrate extracts.

The changes in the solubility of the various protein fractions which occur between the *pre-rigor* state immediately *post mortem* and the *post-rigor* state 24 h. *post mortem* are shown by comparing Helander's values in the footnote to Table I[13] with the values for control sample M.

Since the contribution of elastin to the total OHP present may be assessed at less than 1%,[14] and may indeed be of the order of only 0·15%,[15] the OHP values were taken as a measure of the collagen and its soluble derivatives present in any fraction. After aliquots of the extracts had been taken for estimation of total nitrogen, the remainder of the extracts was dialysed against water to remove non-protein material. The dialysates were concentrated *in vacuo* to convenient volume and hydrolysed by boiling in 8N-hydrochloric acid for 8 h. The hydrolysates were concentrated *in vacuo* to remove most of the acid, neutralised with sodium hydroxide and made to volume for estimation of OHP and tyrosine. By this procedure, from the extraction of 15 g. of muscle containing a total OHP content of 500 μg./g. it was possible by having a final neutralised concentrate of 5 ml. volume to detect the presence of less than 0·5% of the total OHP. At this low volume, the separation of an appreciable insoluble residue of the less soluble amino-acids greatly reduced interference from this source, particularly from tyrosine in the colorimetric determination of OHP.

Insoluble residue.—The insoluble residue remaining after extraction was hydrolysed in 8M-hydrochloric acid for 8 h. and the hydrolysate analysed for total N, OHP and tyrosine.

Trichloroacetic acid-soluble N (TCA-sol. N).—The proteins present in aliquots of the 0·1M-potassium chloride homogenates were precipitated in 10% trichloroacetic acid (TCA) and the filtrates analysed for total non-protein N. The remainder of the filtrates, after extraction with ether to remove TCA, were concentrated and analysed for OHP and tyrosine, in certain cases both before and after acid hydrolysis. The tyrosine values of the extracts usually increased by about 20% after acid hydrolysis. *Total N values* were obtained by the Kjeldahl method.

Hydroxyproline (OHP).—Hydroxyproline was estimated by Neuman & Logan's method[16, 17] which is subject to interference by tyrosine and to inhibition of colour development by the presence of other amino-acids.

In later analyses of similar material, it was found that the Neuman & Logan values uncorrected for interference by tyrosine etc. were equivalent to 80% of the more reliable values obtained by Woessner's method[18] applied to extracts before and after removal of other amino-acids by the Partridge & Elsden procedure.[19]

The OHP values quoted here are the original Neuman & Logan values corrected by the above factor.

Tyrosine.—Values for tyrosine were obtained from the absorption values in 0·2N-sodium hydroxide solution at λ 293. It is realised that values so obtained may not represent accurately the true tyrosine values but it was considered that the values would be sufficiently accurate to serve as a 'tyrosine index' in this preliminary study.

pH.—The pH of homogenates of the samples in water was measured by glass electrode. The tabulated values, 'pH before storage', were obtained by holding representative samples of the muscles at 15–20° for 24 h. *post mortem* during which they passed into *rigor mortis* and reached their lowest, 'ultimate', pH values.

Examination for microbiological sterility

The tissue and any exuded fluid were examined for sterility before being analysed. Fluid, where present, was smeared on a slide, stained by Gram's method and examined. Gross con-

tamination was readily detected in this way. Also, small samples of the tissue, removed aseptically, were inoculated into Robertson's cooked meat medium and incubated at 30° for 5 days. At the end of the incubation period, samples of the medium were checked microscopically for evidence of growth and a loopful from each tube was plated on Hartley's digest agar as a further check on sterility.

Texture observations

In this series of experiments, no physical measurements of texture, involving, for example, shear tests, were made. In some of the later tests, however, certain observations were made on the degree of disintegration of structure in samples homogenised in 0·02M-phosphate buffer in 0·1M-potassium chloride at pH 7·0 in a Marsh-Snow machine under standard conditions.[20] Homogenisation was done at two speeds of the cutting rotor—low at approximately 600 r.p.m. and high at approximately 1800 r.p.m. The low-speed action was just sufficient to break up a sample of fresh muscle into mostly single fibres with a few remaining clumps of fibre bundles. The proportions and dimensions of the fibres and fibrils present in several fields representative of the whole homogenate were observed microscopically.

Results

The results in Tables I and II show the changes which take place in the various fractions of rabbit and bovine muscle during aseptic storage at 37°. Corresponding values for a single sample of bovine muscle held at 5° for 30 days are included for comparison.

Trichloroacetic acid-soluble N (non-protein N)

The TCA-sol. N values are plotted in Fig. 1 and show in both beef and rabbit muscle the accumulation of non-protein N (N.P.N.) resulting from autolysis of protein.

The rather wide scatter of values for the autolytic activity in rabbit muscle is most probably due to sampling errors arising from the need to use composite samples taken at random from different parts of the two whole muscles. It has been shown that there are appreciable differences between different parts of the *longissimus dorsi* muscles in ultimate pH and distribution of fat and protein fractions[21] and similar differences may exist in the distribution of autolytic activity.

Samples from rabbits I and H show anomalous decreases in TCA-sol. N in the interval between 17 and 50 days at 37° whilst simultaneously showing appreciable increases in tyrosine soluble in TCA and OHP soluble in potassium chloride solution (Table I).

The fact that such anomalies were not observed in the bovine samples would support the contention that they are due to sampling errors. The beef samples were all taken from the same section of a very much larger muscle, and consequently would show smaller differences between individual samples.

As shown by the values for the unflamed rabbit samples (Fig. 1, points A and B), the process of flaming does not appear to have any significant effect on the course of production of N.P.N.: further, as shown by points R and S (Fig. 1), there is no difference in this respect between beef samples stored in air and in nitrogen.

In muscle at the normal ultimate pH of 5·5–5·8, the rate of production of TCA-sol. N over the first 10 days at 37° is approximately 20·7 μmole and 10·3 μmole of N/g./day for rabbit and beef respectively.

In muscles from two rabbits treated with adrenaline in which the ultimate pH were 6·4 and 6·8, the rates of production of TCA-sol. N were reduced to approximately 0·5 and 3·6 μmole of N/g./day respectively. On the limited results available, these two rates must be accepted provisionally until further tests are made.

In muscle at pH 5·6–5·8 over 5–6 months at 37°, the TCA-sol. N increased from original values of 10–13% to 37% and 31% of the total nitrogen (TN) for rabbit and beef respectively; consequently, 29% and 22% respectively of the total protein in these muscles had become autolysed.

The curves for the production of tyrosine soluble in TCA were similar in shape to those for

Table I

Changes in various fractions of rabbit muscle (longissimus dorsi) *during aseptic storage at* 37° (*N values as* % *of total N*)

Animal	Storage, days at 37°	pH (1) Before storage (2) After storage	TCA-sol. N (N.P.N.)	Protein-N soluble in			Total sol. protein-N (a)+(b)+(c)	Total N in residue	% of total tyrosine soluble in TCA*	Hydroxyproline (OHP)			TCA-sol. N (N.P.N.) μmol./g.
				0·1M-KCl (a)	0·2M-Na_2HPO_4 (b)	0·1M-citrate (c)				Total μg./g.	Sol. in 0·1M-KCl μg./g.	% of total sol. in 0·1M-KCl	
M (Control)	1 day at 15°	(1) — (2) 5·6	13·0	27·8	23·9	1·7	53·4	28·2	4·1	452	nil	nil	176
A	8·5	(1) 5·6, (2) 5·78	29·1	9·2	—	—	—	61·5	16·1	450	nil	nil	383
	15·5	(2) 5·76	29·1	4·5	—	—	—	61·8	16·7	631	nil	nil	383
B	54	(1) — (2) 5·62	32·2	2·2	—	—	—	61·5	23·4	672	nil	nil	478
	153	(1) — (2) 5·62	37·1	3·0	—	—	—	54·5	32·0	660	<14	<2·1	560
Samples flamed in ethanol													
C (Control)	1 day at 1° Normal flaming	(1) 5·7	13·1	27·6	—	—	—	59·3	5·5	402	nil	nil	178
	Excess flaming	(1) 5·7	13·1	25·9	—	—	—	61·0	5·3	530	nil	nil	178
D (Control)	Normal flaming	(1) 5·85	12·6	—	—	—	—	—	—	—	nil	nil	173
E	19	(1) 5·7, (2) 5·92	25·7	2·5	6·6	0·02	9·1	59·5	19·8	667	73	10·9	375
F	16	(1) 5·8, (2) 6·0	31·4	4·0	—	—	—	63·6	17·4	741	26	3·5	408
	37	(2) 6·0	32·1	2·2	—	—	—	64·5	24·0	574	79	13·8	423
G	61	(1) — (2) 5·68	40·4	1·0	—	—	—	57·5	26·2	685	131	19·1	485
H	17	(1) 5·58, (2) 5·87	31·8	3·2	—	—	—	62·6	19·2	782	27·5	3·5	428
	50	(2) 5·87	30·0	3·2	—	—	—	67·2	25·6	870	105	12·1	405
I	19	(1) 5·82, (2) 5·92	29·9	4·0	—	—	—	61·4	20·8	836	55·5	6·6	405
	45	(2) 6·0	27·8	2·2	—	—	—	69·0	30·0	962	116	12·1	373
J	84	(1) 5·6, (2) 5·86	32·8	1·4	—	—	—	62·3	32·6	849	109	12·9	495
Flamed samples from adrenaline treated rabbits													
K	15	(1) 6·82, (2) 6·64	15·0	9·6	2·06	0·67	12·3	69·9	9·0	479	<9	<1·9	230
L	22	(1) 6·41, (2) 6·40	12·1	4·4	—	—	—	79·5	8·3	480	14·5	3·0	185
	65	(2) 6·49	13·3	3·1	—	—	—	78·0	15·7	545	84·5	15·5	203

Note Fractionation by Helander[13] of total N in rabbit muscle immediately *post mortem*. N values as % of total N: Myofibrillar proteins, 52·3; sarcoplasmic proteins, 31·8; stroma proteins, 5·5; N.P.N., 10·4

* In these samples tyrosine was estimated in the unhydrolysed extracts. The average of the total tyrosine values of the *longissimus dorsi* of the individual animals was 17·5 mg./g.

Table II

Changes in various fractions of bovine muscle (longissimus dorsi) *during aseptic storage at* 37° (*N values as % of total N*)

Animal	Storage, days at 37°	pH (1) Before storage (2) After storage	TCA-sol. N (N.P.N.)	Protein-N soluble in			Total sol. protein-N (a)+(b)+(c)	Total N in residue	% of total tyrosine soluble IN TCA†	Hydroxyproline (OHP)		TCA-sol. N (N.P.N.) μmol./g.
				0·1M-KCl (a)	0·2M-Na_2HPO_4 (b)	0·1M-citrate (c)				Total μg./g.	Sol. in 0·1M-KCl μg./g.	
N (Control)	2 days at 0°	(1) 5·61, (2) 5·61	10·9	20·0	7·4	1·31	28·7	58·0	10·9	—	—	139
	3 days at 0°	(1) 5·61, (2) 5·61	10·9	18·8	7·4	1·66	27·9	57·5	—	—	—	
Samples stored in air												
O	10	(1) 5·78, (2) 5·78	17·0	7·6	5·5	0·3	13·4	63·4	10·5	650	nil	223
	20	(1) 5·78, (2) 5·78	17·8	7·8	3·8	0·3	12·9	63·0	12·7	810	nil	240
P	46	(1) 5·70, (2) 5·86	23·3	7·4	3·0	0·3	10·7	64·5	17·3	710	nil	293
	112	(1) 5·70, (2) 5·95	27·7	5·0	—	—	—	64·0	25·8	925	nil	362
Q	63	(1) 5·6, (2) 5·85	25·6	4·8	—	—	—	69·7	23·8	836	nil	326
	172	(1) 5·6, (2) 5·78	31·4	4·4	1·6	0·1	6·1	60·8	34·9	735	<5	390
Samples stored in nitrogen												
R	2	(1) 5·64, (2) 5·74	12·8	13·4	4·9	—	—	69·0	11·8	995	nil	151
	8	(2) 5·74	17·5	10·8	5·5	—	—	68·6	15·7	1110	nil	214
S	2	(1) 5·45, (2) —	12·8	12·5	5·1	—	—	67·7	11·4	825	nil	163
	10	(1) 5·45, (2) —	18·7	10·5	5·5	—	—	67·0	15·7	840	nil	242
	30	(1) 5·45, (2) 5·76	24·2	10·4	3·9	—	—	61·4	20·9	790	nil	315
	30 days at 5°	(1) 5·45, (2) 5·66	14·8	19·9	10·0	—	—	52·3	14·8	842	nil	189

† In these samples tyrosine was determined in the TCA extracts after acid hydrolysis. The average of the total tyrosine values of the *longissimus dorsi* of the individual animals was 17·7 mg./g.

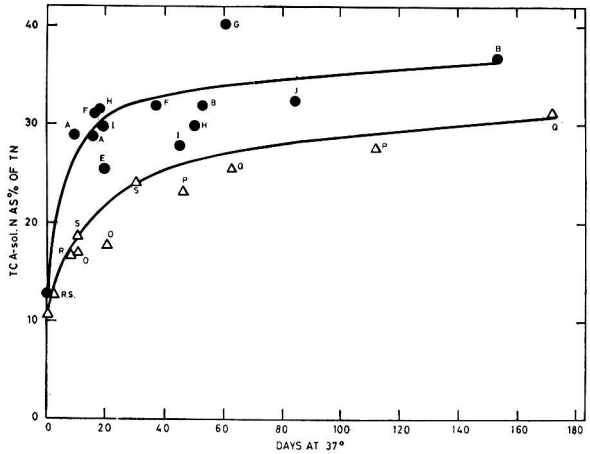

FIG. 1.—*Production of TCA-sol. N in muscle during aseptic storage at 37°*
Letters indicate individual animals
●——● Rabbit muscle △——△ Bovine muscle

the production of TCA-sol. N in both bovine and rabbit muscle. The tyrosine present accounted, however, for only 2–4% of the total TCA-sol. N.

No traces of OHP were found in any of the TCA extracts.

Changes in solubility of the protein fractions

The proportion of soluble protein decreased with duration of storage at 37°, e.g., in samples of one rabbit, E, the total soluble protein N had decreased to 9·1% of the TN after 19 days. The protein-N soluble in 0·1M-potassium chloride decreased in most of the rabbit samples to 2·5–4·0% of the TN in 19 days at 37°.

In samples of bovine muscle, the rate of decrease in solubility was lower, e.g., in samples from animal S after 30 days more than 14·3% of the TN was still present as soluble protein N, and in samples from animal P after 46 days, 10·7% of the TN remained as soluble protein N. The protein-N soluble in 0·1M-potassium chloride was 10·4% in samples from animal S after 30 days and 4·4% in samples from animal Q even after 172 days at 37°.

In the single sample of bovine muscle stored at 5° for 30 days, there was no significant change in the proportion of protein-N soluble in 0·1M-potassium chloride, but there were appreciable increases in the protein-N soluble in phosphate and in the N soluble in TCA. These changes were similar to those observed by Locker.[4]

Soluble fractions containing OHP (soluble collagen derivatives)

In unflamed samples, only after 5–6 months' storage at 37° was there any evidence of the presence of OHP in any of the soluble fractions. In rabbit B after 153 days and in beef animal Q after 172 days doubtful traces of less than 14 μg. OHP were present in the 0·1M-potassium chloride extracts.

In all the flamed samples of rabbit muscle, however, large proportions of OHP were present in the 0·1M-potassium chloride extracts amounting in one animal (G) to 19·1% of the total OHP present. The values for soluble OHP are plotted in Fig. 2 which shows the continuous formation of soluble collagen derivatives at a rate of 2·0–2·6 μg. of OHP/g./day. In the muscle at pH 6·4 from the adrenalised rabbit W, although the rate of production of TCA-sol. N is very low com-

pared with the rate at pH 5·6–5·8, the rate of formation of soluble derivatives from collagen is reduced by only about half to 1·3 μg. of OHP/g./day.

Texture changes

Fig. 3 demonstrates the difference between homogenates of control rabbit muscle H (pH 5·6) and of muscle from an adrenalised rabbit L (pH 6·4) after storage at 37° for 50 days and 65 days respectively. (It must be noted that these homogenates cannot be compared with the homogenates of bovine muscle referred to below since they were prepared under different conditions.) The control sample (H) became disintegrated into short sections of fibres and fibrils whereas the adrenalised muscle (L) produced a mass of long fibres showing longitudinal splitting at the ends and very few single fibrils.

In the control sample, 21% of the protein N

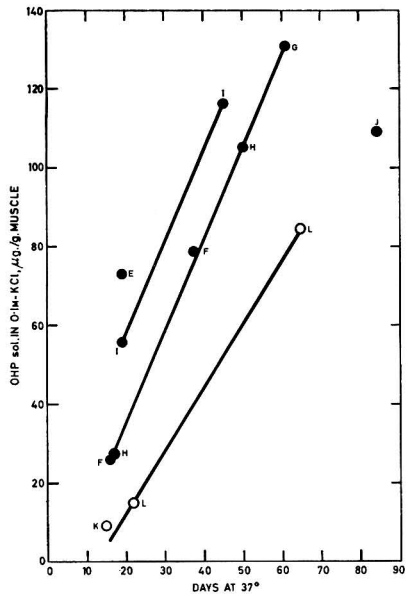

FIG. 2.—*OHP in* 0·1M-*KCl extracts of flamed rabbit muscle after aseptic storage at* 37°
Letters represent individual animals
○ Animals K and L treated with adrenaline

had become soluble in TCA whereas in sample L less than 1% had become soluble. At the same time, in both samples an appreciable proportion of a soluble collagen fraction had been formed equivalent to 84·5–105 μg. of soluble OHP/g. of muscle (Table I).

It would seem, therefore, that under these conditions, increase of pH from 5·6 to 6·4 caused a very large reduction in the rate of proteolysis of intracellular protein but had a much smaller effect on the production of soluble OHP, reducing the rate of this activity by not more than half. The degree of disintegration of the tissue during homogenisation would therefore appear to be related to the degree of intracellular proteolysis which has taken place.

Observations on the dimensions of fibres and fibrils in homogenates of bovine muscle after aseptic storage at 37° and 5° for 30 days are given in Table III and Figs. 4–6.

The degree of breakdown of the tissue structure was far greater after storage at 5° than at 37°, even although at 37° approximately 14·9% of the original protein-N had become soluble in TCA as compared with not more than 4·4% in the samples held at 5°. There was no evidence in either sample of any change in the solubility of the collagen fraction.

Samples from another beef animal after 19 days at 5° and 37° gave similar differences. After storage at 5° the fibres became broken transversely to give short sections which eventually produced short sections of fibrils; after storage at 37°, the fibres showed mainly longitudinal cleavage resulting in long sections of both fibres and fibrils.

It would seem therefore that, although during storage at 37° there is a greater degree of autolysis of intracellular proteins, the fibres and fibrils become structurally stronger probably by denaturation of the proteins, during storage over a relatively long period at the higher temperature.

Wilson *et al.*[22] found that beef held for 24 h. at 43° was as tender after cooking as beef which had been held for 14 days at 2°. They observed however that after a further 16 h. at 43°, the beef became less tender. This tendency to become tougher with time at higher temperatures would explain the present observed differences in texture of homogenates of samples after storage at 5° and 37°. An explanation of the toughening process will have to await further observations on the effect of these conditions on the several protein fractions involved.

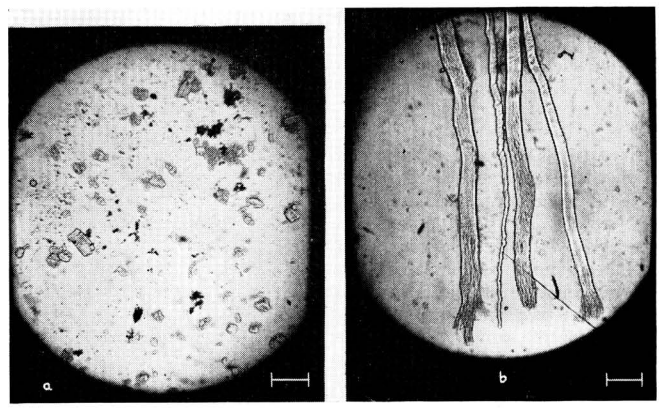

FIG. 3.—*Photomicrographs of homogenates of samples of rabbit muscle after aseptic storage at 37°*
A Muscle from untreated rabbit H (pH 5·6) after 50 days at 37°
B Muscle from adrenalised rabbit L (pH 6·4) after 65 days at 37°
(scale represents 200 μ)

Homogenates of samples of bovine muscle held for periods of up to 112 days at 37° (Sample P) showed no significant differences from the above findings with samples after 30 days, although further proteolysis of the intracellular proteins had occurred. There were indications however that after 172 days (Sample Q) the structure had weakened and the homogenate contained an appreciable proportion of fibrils. This tendency requires to be confirmed by further tests.

In all stored samples without exception, even after 172 days at 37°, the basic structure of the fibres and fibrils as shown by the presence of cross striations remained intact.

Discussion

Although it is difficult to make a close comparison owing to the different experimental conditions and techniques employed, the present results agree broadly with the results of Zender *et al.*[2] and Radouco-Thomas *et al.*[3] with regard to the general course of autolysis. These workers observed almost 100% degeneration of the fibres in lamb muscle stored at 25° for 100 days and in rabbit muscle at 38° for 14 days. The muscle structure in these cases had become so weakened by autolysis that samples fell to pieces on submerging in 25% glycerol–0·5M-potassium chloride solution. No such advanced degree of degradation was observed in the present study with rabbit and ox muscle stored at 37°. The above workers however observed very much less

Table III

Dimensions of fibres and fibrils present in greatest number in homogenates of sterile stored bovine muscle (longissimus dorsi)

Storage history, days (° c)	Dimensions of fibres and fibrils in homogenates		
	Length (μ)		Diameter (μ)
	Fibres	Fibrils	Fibres
		Low speed	
2 (−20°)	650–1300	—	200–260
30 (37°)	250–430	—	43–170
30 (5°)	50–170	—	14–86
		High speed	
2 (−20°)	450–860	170–700	150–180
30 (37°)	170–250	66–110	—
30 (5°)	—	40–170	—

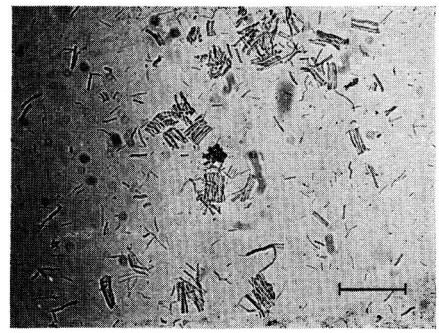

FIG. 4.—*Photomicrograph of homogenate (low speed) of bovine muscle S after aseptic storage at 5° for 30 days*
Field shows large proportion of short fibre sections in background of short fibrils (cf. Table III)
(scale represents 200 μ)

degeneration in pig and sheep muscle after storage for 35 days at 38° amounting to only 25% as compared with 100% for lamb and rabbit muscle. It would seem therefore that large differences exist in the susceptibility of muscle tissue from different species to aseptic autolytic degradation. On the basis of the limited number of results available, the disparity between the two studies in this connexion may most probably be due to differences in the autolytic activity of the muscles from animals of different breeds and/or of different stages of maturity.

In this present study, the basic structure of the fibres and fibrils as shown by the presence of cross striations remained intact even after 172 days at 37°. The substrate for proteolysis would therefore appear to be mainly the sarcoplasmic protein fraction. The highest level of proteolysis observed in unflamed muscle samples after storage at 37° corresponded to 27·7% and 23% respectively of the original total protein-N for rabbit sample B after 153 days and beef sample Q after 172 days. The total sarcoplasmic protein fraction from rabbit[13] and bovine muscle[23] is equivalent to 35% of the total protein-N. It is possible therefore on the basis of these values that the whole of the increase in TCA-sol. N is derived from the sarcoplasmic proteins.

The tests with muscle from adrenalised rabbits showed that the production of TCA-sol. N was at least 6 times and possibly 40 times greater at pH 5·6–5·8 than at pH 6·4–6·8. It is difficult to correlate this relationship between pH and activity with published observations. Snoke &

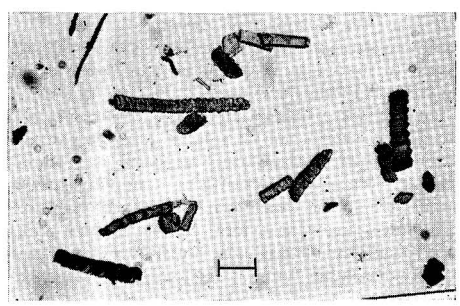

FIG. 5.—*Photomicrograph of homogenate (low speed) of bovine muscle S after aseptic storage at 37° for 30 days*
Field shows large proportion of fibre sections with relatively few fibrils (cf. Table III)
(scale represents 200 μ)

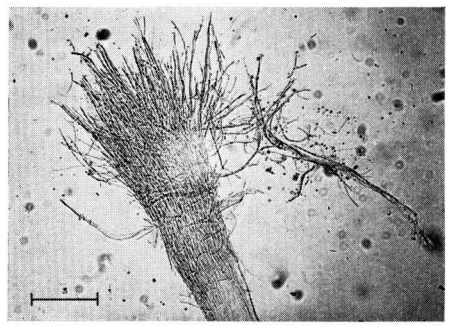

FIG. 6.—*Photomicrograph of fibre in homogenate represented in Fig. 5 showing typical longitudinal disintegration* (scale represents 200 μ

Neurath investigated a proteolytic enzyme fraction from rabbit muscle which had optimum activity at pH 4 and low activity in the range 5·3–6·3 with haemoglobin as substrate.[24] More recently Sliwinski *et al.*[7] have separated from ox muscle three enzyme fractions with optimum proteolytic activity at pH 4–5, 8–9 and 10 using as substrate the protein fraction from ox muscle soluble in 2% potassium chloride. Koszalka & Miller[8] found that proteolysis of a homogenate of rat muscle in 2% potassium chloride showed a peak of relatively low activity at pH 3·5–4 and a peak of much greater activity at pH 8·5–9·0.

It is clear that in unflamed, sterile rabbit and beef muscle no change takes place in the connective-tissue protein collagen over 6 months at 37° to make it significantly soluble in 0·1M-potassium chloride. In the few cases where extraction with phosphate at pH 8·9 and citrate at pH 3·8 were done after potassium chloride extraction, there was no evidence of the presence of any soluble collagen in the extracts. In rabbit and beef muscle after 5–6 months at 37°, there is only a doubtful trace of soluble collagen present in the potassium chloride extracts.

The situation with regard to flamed rabbit muscle is quite different. In this case, during flaming some change has occurred in the enzyme system and/or the state of the collagen which allows continuous solubilisation of the collagen to take place over a period of at least 60 days at 37°. At this point in one sample (G) 19·1% of the total collagen as measured by the OHP present was soluble in 0·1M-potassium chloride. Even in sample (L) from the adrenalised rabbit, 15·5% of the total OHP was present in the potassium chloride soluble fraction. If this is due to enzyme action, it means that the enzyme is more active at the higher pH than the enzyme which hydrolyses the sarcoplasmic proteins.

Control samples analysed immediately after flaming (samples C and D) showed no increase in TCA-sol. N, no decrease in the solubility of the proteins in 0·1M-potassium chloride and no evidence of any soluble OHP in the latter extracts. The rise in temperature in the interior to not more than 37° was not sufficient to cause inactivation of enzymes. On the results available, it would seem therefore that the formation of soluble collagen fractions in samples of flamed muscle is caused by enzymic action which has been triggered off in some way during the flaming process. On the other hand, more recent tests with beef samples heated at 65–70° for 20–25 min. or at 95–100° for 60 min. have shown that during subsequent aseptic storage at 37°, there is continuous conversion of the collagen to a form soluble in 0·1M-potassium chloride.[10] This change cannot be regarded as enzymic and is most probably a purely physical conversion of heat denatured collagen to a more soluble form.

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166 and iodine value 62, whilst Berger[2] reports corresponding values of 199 and 117. Since no satisfactory examination of this seed oil has been reported and as the oil is said to behave unusually in certain undefined respects,[3] it was decided to examine it by chromatographic methods.

Experimental and results

The oval seeds contain kernels (74%) which were crushed and extracted with light petroleum (b.p. 40–60°) in a Soxhlet apparatus. The resulting oil (28·1% of the kernels), of iodine value 66·5, was hydrolysed, and unsaponifiable material (0·8%) and mixed acids free of unsaponifiable material were recovered. The latter had iodine value 69·8 and equivalent weight 288·6.

The mixed acids, (*a*) without other treatment, (*b*) after hydrogenation, and (*c*) after ozonolysis were examined by reverse-phase chromatography as previously described[4] with the results given in Tables I and II.

Table I

Chromatography results (mole-%) on paraffin columns

Treatment of mixed acids	Load, mg.	Recovery, %	Eluting solvent (% acetone) 43	53	62	67	73	78	83
Hydrogenation	20·8	83	0·7	0·5	0·7	17·0	68·9	4·9	4·1, 3·1
None	20·3	96	1·4	0·2	4·8, 6·9	72·6	7·5	2·5	4·1
Ozonolysis	38·2	—	?	0·5	0·9	12·5	3·4	2·7	3·3, 1·7

Table II

Component acids (mole- and wt.-%) to the nearest 0·5%

Acid	10 : 0*	12 : 0	14 : 0	16 : 0	16 : 1	18 : 0	18 : 1	18 : 2	18 : 3	20 : 0	20 : 1	22 : 0	24 : 0
Mole-%	1·0	0·5	1·0	12·5	4·5	3·5	59·5	6·5	tr.	2·5	3·0	3·5	2·0
Wt.-%	0·5	0·5	0·5	11·5	4·0	3·5	60·0	6·5	tr.	3·0	3·5	4·0	2·5

* These figures indicate the number of carbon atoms and the number of double bonds respectively in the acids.

Values calculated for mixed acids of this composition: iodine value 72·4, saponification value 280·4.

Oleic acid was identified as *erythro*-9,10-dihydroxystearic acid (m.p. and mixed m.p. 129–130°), and linoleic acid as 9,10,12,13-tetrabromostearic acid (m.p. and mixed m.p. 113–114°). Bromination also yielded a little 9,10,12,13,15,16-hexabromostearic acid (m.p. and mixed m.p. 181–182°) indicating the presence of traces of linolenic acid. The eicosenoic acid is probably the familiar Δ^9-isomer since hendecanoic acid was shown, by gas–liquid chromatography, to be a product of ozonolysis.

In the chromatographic examination of the mixed acids, the elution curve with 62% acetone had two peaks. This has been observed on other occasions and has been shown to be due to a partial separation of hexadecenoic and linoleic acids. After hydrogenation and after ozonolysis, when only saturated acids are present, the elution curve with 83% acetone also showed two peaks and these are considered to be due to the saturated C_{22} and C_{24} acids.

Qualitative evidence for the presence of all the acids listed in Table II was obtained by gas–liquid chromatography using Apiezon L columns in a Pye Argon chromatograph. There were also indications of very minor amounts of the odd acids between C_{17} and C_{23}.

Discussion

T. cearensis belongs to the Caesalpinoideae sub-family of the Leguminosae and the component acids of several other species of this sub-family are reported by Hilditch.[5] Several of these contain minor amounts of the higher saturated acids (C_{20}–C_{24}) as does this seed oil, but they are more unsaturated than the amburana oil, the content of linoleic acid (36–63%) exceeding that of oleic acid (21–34%) in all cases. In amburana oil linoleic acid (6·5%) is only a minor component. Other differences are the presence in amburana oil of very small amounts of the C_{10}, C_{12} and C_{14} saturated acids and of small amounts of the C_{16} and C_{20} monoethenoid acids, although these differences may result from the more refined analytical methods now available.

Acknowledgments

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VEGETABLE OILS. XII.*—*Vernonia* Seed Oils

By R. C. BADAMI and F. D. GUNSTONE

A number of *Vernonia* seed oils have been examined by reverse-phase chromatography: all contain oxygenated acid(s) (8–90%) which is mainly epoxyoleic acid.

Introduction

A natural epoxy acid was first recognised in a sample of *Vernonia anthelmintica* seed oil containing 72% of (+)-12,13-epoxyoleic acid.[1] This acid has since been recognised in several seed oils derived from three different plant families, and five other natural long-chain epoxy acids—15,16-epoxylinoleic acid, (−)-12,13-epoxyoleic acid, 9,10-epoxyoctadec-12-enoic acid, and *cis*- and *trans*-9,10-epoxystearic acid—have now been recognised. Details are summarised in Table I.

Seed oils rich in epoxy acids are of potential interest as replacements for synthetic epoxy compounds used as stabilisers for plastic materials[2] and also as starting materials for preparation of other long-chain compounds.[3] It is of interest, therefore, to examine other seed oils which might contain epoxy acids. This paper reports the examination of seed oils derived from six *Vernonia* species, four of which have not previously been reported.

Values quoted in Table I show that *Vernonia* oils are unusual in that, whilst two species generate a high proportion of epoxyoleic acid in their seed oils, five other species of this same genus produce little or no epoxy acid. All of the four species hitherto unexamined and reported here, contain oxygenated acids.

Experimental and results

V. anthelmintica seeds[1] and *V. camporum* seeds[4] were available from previous investigations. Seeds of *V. cinerea* were obtained from Poona (India) and seeds of *V. colorata*, *V. amygdalina*, *V. biafrae* and *V. nigritiana* from Sierra Leone: only small quantities were available.† Most of the seeds were attached to feathery fibrous matter which was removed when this was easily possible (*V. nigritiana*, *V. amygdalina*, *V. cinerea*); otherwise (*V. biafrae*, *V. colorata*) seed and attached fibre were extracted. The seeds were crushed and extracted with light petroleum (b.p. 40–60°) in the usual manner. Mixed acids free of unsaponifiable material were then isolated from the oils under conditions which would not hydrolyse epoxy groups, viz., hydrolysis with

* Part XI: preceding paper
† These seeds were obtained in 1958 and examined 3 years later

Table I

Natural occurrence of long-chain epoxy acids[a]

Plant family	Species	Epoxy acid[b]	Content, wt.-%	Reference
Compositae	*Vernonia anthelmintica*[c]	(+)-12,13-epoxyoleic	72–80%	1, 8
,,	*V. colorata*	,,	high	9
Euphorbiaceae	*Cephalocroton cordofanus*	,,	62	7
,,	*C. peuschelii*	,,	72	4
Onagraceae	*Clarkia elegans*	,,	14	10
Malvaceae	*Hibiscus esculentus*	(−)-12,13-epoxyoleic	3	9
,,	*H. cannabinus*	,,	5	11
,,	*Malope trifida*	,,	9	12
,,	Ten other malvaceous spp.	,,	0·5–7	12
Compositae	*Chrysanthemum coronarium*	9,10-epoxyoctadec-12-enoic	3–16	10
Cruciferae	*Camelina sativa*	15,16-epoxylinoleic	1	13
Compositae	*Tragopogon porrifolius*	9,10-epoxystearic	3	14
—	Various plant rusts	,,	1–74	15
Oleaceae	Oroju oil	*trans*-9,10-epoxystearic	4	16

[a] There is evidence of other unidentified epoxy acids in several seed oils.[6, 17, 18]
[b] All are *cis*-epoxy acids except where otherwise stated.
[c] Other *Vernonia* oils are reported to contain little or no epoxy acid. These include *V. camporum*, *V. deppeana*, *V. fasciculata*, *V. baldwini* and *V. missurica*.[4, 17, 18]

cold 1N-alcoholic potassium hydroxide and, after extraction of the unsaponifiable material, acidification with an ion-exchange resin (Zeocarb 225).[4]

Mixed esters, prepared from the acids by reaction with diazomethane, were examined by gas–liquid chromatography with a Pye Argon Chromatograph fitted with 2½% Apiezon column (temp. 200°; flow-rate 33 ml. per min.). On such a column methyl epoxyoleate has a 'carbon number'[5] of 19·1 and a large peak of this value was observed with esters derived from *V. anthelmintica*, *V. amygdalina*, *V. cinerea* and *V. colorata*, a smaller peak with the esters from *V. nigritiana*, and no peak at all with the esters from *V. biafrae* and *V. camporum*. The five samples having a peak of 'carbon number' 19·0–19·1 also show minor peaks running just before (18·7–18·9) and just after (19·3–19·4) the main peak. These probably result from small amounts of dienoic and saturated C_{18}-epoxy compounds. Some of the material eluted after these peaks may be dihydroxyoleate.

Morris & Holman[6] have described a spectroscopic method of estimating epoxides depending on the increased absorption at 2·795 μ after the conversion of epoxides to chlorohydrins by treatment with ethereal hydrogen chloride. This method was found less accurate than was claimed by Morris & Holman, although the results obtained are in line with those obtained by chromatographic investigation (Table II).

Table II

Vernonia seed oils

Vernonia spp.	*anthelmintica*	*amygdalina*	*colorata*	*cinerea*	*biafrae*	*nigritiana*	*camporum**
Oil in seeds, %	26·9	5·0	3·1	3·8	1·6	7·6	8·4
Unsaponifiable, %	8	29	45	73	68	18	2·5
Epoxy acid, % (infra-red determination)	68	50	14	19	nil	5	nil
Component acids (mol.-%) extracted by :							
43% acetone	1	4	20	5	8	4	0
53% acetone	77	55	14	24	3	2	0
Component acids (wt.-%)							
Myristic	1	1	9	8	6	2	0
Palmitic	5	8	11	23	21	18	14
Hexadecenoic	0	0	5	0	10	0	0
Stearic	2	5	6	8	8	8	9
Oleic	1	6	12	4	15	19	22
Linoleic	9	20	15	22	21	43	55
Arachidic	1	1	2	3	2	1	0
Behenic	1	1	2	4	4	1	0
Oxygenated acid(s)	80	58	38	28	13	8	0

* These values, apart from the infra-red determination of epoxy acid, are taken from reference 4.

The main quantitative examination of these oils was by the reverse-phase chromatographic procedure previously described.[4] The mixed acids were examined (*a*) without further treatment, (*b*) after acetylation and hydrogenation, and (*c*) after ozonolysis. In calculating the results, acids lower than C_{14} are assumed to be absent and all acids eluted with 43% and 53% aqueous acetone, whether appearing under one or more peaks, are classed together as oxygenated acids. This must be mainly epoxyoleic acid and is probably accompanied by dihydroxyoleic acid. The results are summarised in Table II. Because of the small quantity of material available, iodine values and saponification equivalents could not be measured. The lack of this latter value necessitated a modification in the calculation of the ozonised acids; C_{20} and C_{22} unsaturated acids were assumed to be absent (or insignificant) and the proportion of stearic acid after ozonolysis was adjusted to agree with that indicated in the mixed acids, the proportion of other saturated acids remaining after ozonolysis could then be calculated.

Discussion

The oil content of *Vernonia* seeds seems to be generally low and it is fortunate that the oil with the highest proportion of epoxy acid (*V. anthelmintica*) comes from the seed of highest oil content. The small extract obtained from many of the *Vernonia* seeds sometimes contains a high proportion of unsaponifiable material which further reduces its potential value. *V. anthelmintica* remains the richest source of epoxy acids although two *Cephalocroton* species, and possibly other unexamined *Cephalocroton* oils, deserve further attention:

	Oil content, %	Epoxyoleic acid, wt.-%
V. anthelmintica[1, 8]	27	78–80
C. cordofanus[7]	33–42	62
C. peuschelii[4]	30	72

It is considered that the major oxygenated acid in the *Vernonia* oils is most often epoxyoleic acid, although this is frequently accompanied by a more polar acid, probably dihydroxyoleic acid. Comparison of the epoxy acid content determined by the method of Morris & Holman,[6] the total content of oxygenated acids, and the proportion of the mixed acids eluted from a paraffin column with 43% acetone (probably dihydroxyoleic acid) and with 53% acetone (epoxyoleic acid) suggests that the sample of *V. colorata* seed oil contained a high proportion of dihydroxy-acid and that the oxygenated acid in *V. biafrae* seed oil is largely dihydroxy-acid.

The *Vernonia* soils containing smaller proportions of oxygenated acids generate rather more linoleic acid and also, to a lesser extent, more oleic, palmitic and myristic acids. This may indicate that the epoxyoleic acid is derived from linoleic acid, or *vice versa*, or that both acids are formed from a common precursor.

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COMPOSITION OF EDIBLE WILD PLANTS OF LEBANON

By J. W. COWAN, A. H. SAKR, S. B. SHADAREVIAN and Z. I. SABRY

Proximate analysis, β-carotene content and essential amino-acid pattern were determined on various wild plants frequently consumed in Lebanon. In most of the plants studied, ash values were higher than those reported in other parts of the world. The limiting amino-acid was lysine; its availability was not affected by traditional methods of preparation. As normally eaten, these plants supply a large portion of the vitamin-A requirement.

Introduction

Limited surveys among school children[1] and rural families[2] in Lebanon have revealed that, during the winter and spring months, various wild plants constitute a significant portion of the diet in many rural and urban communities. These wild plants grow abundantly in rural areas and many are available in both city and village vegetable markets. Since information is lacking on their nutritive value, the present study was undertaken to collect, identify and determine the composition and the mode of consumption of the more important of these plants.

Experimental

Single samples for identification and analysis were either purchased from the vegetable market or picked where growing.

Methods of analysis

The analyses for moisture, protein, ether extract, crude fibre and ash were performed in duplicate according to the established A.O.A.C. methods.[3] Nitrogen-free extract (N.F.E.) was calculated by difference.

Amino-acids were determined microbiologically. Except for the determinations of tryptophan and tyrosine where alkaline hydrolysates[4] were used, assays were carried out on hydrolysates prepared by refluxing the samples with 6N-hydrochloric acid for 24 h. *Leuconostoc mesenteroides* P-60 (ATCC No. 8042) was used as the assay organism for lysine, phenylalanine, tyrosine and cystine; *Streptococcus lactis* R (ATCC No. 8043) for threonine, tryptophan and methionine; *Lactobacillus arabinosis* 17–5 (ATCC No. 8014) for valine, leucine and isoleucine. The basal media employed were those described by Lyman and associates[4–8] for all the amino-acid assays except for those of tyrosine and cystine in which the media recommended by the A.O.A.C.[3] were used. In all assays, the lactic acid produced was titrated with 0·1N-sodium hydroxide using bromthymol blue as indicator.

'Available lysine' was determined by the 2,4-dinitrofluorobenzene (DNFB) method of Carpenter.[9] Where the effect of preparation on lysine availability was studied, the plants were prepared according to traditional recipes.

Table I

Proximate analysis and mode of consumption of edible wild plants of Lebanon

(results as g. per 100 g. of edible portion)

English name	Botanical name	Arabic name	Edible portion	Moisture	Protein	Ether extract	Crude fibre	Ash	N-free extract	Mode of consumption
Amaranth	*Amaranthus angustifolius* Lam. (*A. sylvestris*) Desf.	Shadokh hindi	Leaves	84·4	5·0	0·8	1·4	2·7	5·7	Cooked
Arum	*Arum* sp.	Luf ga'd	,,	90·1	2·2	0·7	1·1	1·1	4·9	,,
Chickweed, common	*Stellaria media* (L.) Vill.	Hashish el qirar	,,	91·7	1·2	0·2	1·7	1·6	3·6	,,
Chicory, common	*Cichorium intybus* L.	Hindbeh	,,	90·6	2·4	0·4	1·2	1·6	3·8	,,
Crown daisy	*Chrysanthemum coronarium* L.	Mandiliyah	,,	89·5	2·8	0·5	1·5	1·8	3·9	,,
Dock	*Rumex dentatus* L.	Hummaydah-el-tabkh	,,	89·5	3·0	0·5	1·3	1·8	3·9	,,
Eryngo	*Eryngium creticum* Lam.	Qiras'nah zarka	Leaves and stalks	88·7	2·3	0·3	2·1	2·2	4·4	Raw or pickled
Fennel	*Foeniculum vulgare* Mill.	Shumarah	Leaves and stalks	80·5	4·2	0·8	2·7	2·7	9·1	Raw or cooked
Goat's beard	*Urosperum picroides* (L.) F. W. Schmidt	Aby-warakol-aridah	Leaves	91·4	1·6	0·3	1·7	1·3	3·7	Cooked
Gold-of-pleasure	*Rapistrum rugosum* (L). All.	Hharrah	,,	85·6	4·1	0·3	2·0	2·8	5·2	Raw or cooked
Gundelia	*Gundelia tournefortii* L.	A'koub	Leaves and stalks	94·7	1·1	0·1	1·1	0·9	2·1	Cooked
Hawk's-beard, Palestine	*Cymboseris palaestina* Boiss.	—	Leaves	92·8	1·2	0·5	1·2	1·4	2·9	,,
Hawk's-beard	*Crepis aspera* L.	—	,,	88·2	1·9	0·7	1·9	1·1	6·2	,,
Hawkweed	*Crepis reuteriana* Boiss.	Khass-el-hajal	,,	88·2	1·3	0·5	2·3	1·9	5·8	,,
Hyssop	*Origanum syriacum* L. (*O. maru* L.)	Za'tar	,,	73·1	4·0	2·7	3·8	2·5	13·9	Raw or cooked
Inula	*Inula crithmoides* L.	Hashishet-el-baher	,,	91·0	0·9	0·3	0·7	3·3	3·8	Pickled
Lettuce, tuberous	*Lactuca tuberosa* Jacq.	Khass-el-helou	,,	89·7	1·4	0·8	1·6	1·5	5·0	Cooked
Mallow	*Malva montana* Forsk.	Khubbazi	Leaves and stalks	84·3	5·0	0·8	2·0	2·4	5·5	,,
Old-man's beard	*Tragopogon hybridum* L. (*Geropogon glaber* L.)	—	Leaves	75·1	3·2	1·2	6·5	1·8	12·2	Raw
Pea, wild	*Lathyrus digitatus* (M.B.) Fiori (*Orobus sessilifolius* Sibth. et Sm.)	Bisillah barri	Seeds	57·8	10·8	0·3	5·7	1·2	24·2	Raw or cooked
Poppy, Syrian	*Papaver syriacum* Boiss. et Bl.	Shaar-el-arous	Leaves	87·0	3·9	0·6	1·6	1·8	5·1	Cooked
Purslane	*Portulaca oleracea* L.	Riglah	,,	91·8	2·5	0·3	0·9	1·8	2·7	Raw or cooked
Shepherd's needle	*Scandix iberica* M.B.	Mushter ra'i	Leaves and stalks	90·5	1·2	0·4	2·0	1·9	4·0	Raw or cooked
Stork's-bill	*Erodium moschatum* (Brum. f.) L'Her.	Musaykah	Leaves	88·9	2·5	0·5	1·5	1·7	4·9	Cooked
Thistle, star	*Centaurea pallescens* Del.	Ad-dardar-iyah	Leaves and stalks	91·0	2·4	0·2	1·3	1·4	3·7	,,
Thyme	*Thymus capitatus* (L.) Hoffn. et Link	Za'tar farisi	Leaves	78·7	2·9	0·7	2·8	2·0	12·9	Raw or pickled
Truffles	*Terfezia claveryi* Chat.	Kam'ah	Spores	75·5	4·4	1·4	2·4	1·2	15·1	Cooked
Vetch, hairy, yellow	*Vicia hybrida* L.	Foul ba'rri	Seeds	63·0	12·1	0·7	2·3	1·3	20·6	Raw or cooked
Viper's-grass	*Scorzonera parviflora* Jacq.	Khass-el-kilab	Leaves	81·4	4·9	1·0	2·6	2·7	7·4	Cooked
Wart-cress	*Coronopus procumbens* Gilib. (*C. squamatus* Forsk. Aschers.)	Rashad barri	,,	88·2	2·8	0·3	2·0	1·7	5·0	,,
Wood sorrel	*Oxalis cernua* Thunb.	Hhummayda	Leaves, leaf stalks, flower stalks	91·6	0·9	0·5	1·5	1·1	4·4	Raw or cooked

Table II

Content of essential amino-acids in eight edible wild plants of Lebanon

(results as mg. per g. of N)

Common name	Lysine	Phenyalanine and tyrosine	Cystine and methionine	Tryptophan	Threonine	Valine	Isoleucine	Leucine
Amaranth	175	296	208	110	236	333	271	229
Arum	153	333	213	149	179	389	335	321
Chicory, common	134	290	184	129	180	368	314	319
Eryngo	121	280	168	113	156	344	287	291
Hyssop	122	236	143	100	201	275	194	222
Inula	144	287	166	145	272	360	254	270
Mallow	148	243	118	108	210	404	234	269
Thyme	142	331	188	128	173	345	321	295
FAO Pattern	270	180	90	90	180	360	270	306

Table III

'Available lysine' in raw and prepared edible wild plants of Lebanon

(results as mg. per g. of N)

Common name	Total [a]	'Available' [b] Raw	'Available' [b] Prepared [c]
Arum	153	174	164
Chicory, common	134	140	139
Eryngo	121	111	117 128 [d]
Hyssop	122	151	150
Inula	144	126	128 [d]
Mallow	148	170	156
Shepherd's needle	—	142	155
Wood sorrel	—	118	126
Dock	—	225	209
Thistle, star	—	124	121
Stork's-bill	—	115	115

[a] Determined microbiologically.
[b] Determined by the DNFB method.
[c] Samples were prepared by cooking except where otherwise indicated.
[d] Sample was pickled in vinegar.

Table IV

Carotene content and vitamin A potency of various edible wild plants of Lebanon

(results per 100 g. of edible portion)

Common name	β-Carotene, μg.	Vitamin A potency, i.u.
Amaranth	5400	9000
Chickweed, common	600	1000
Chicory, common	2000	3300
Crown daisy	2300	3800
Dock	1900	3200
Gold-of-pleasure	1900	3200
Gundelia	10	20
Hawk's-beard	2900	4800
Hyssop	5600	9300
Inula	600	1000
Mallow	3500	5700
Old-man's-beard	4800	8000
Purslane	1300	2200
Shepherd's needle	1100	1800
Stork's-bill	4200	7000
Truffles	0	0
Viper's-grass	2200	3700
Wart-cress	1300	2200
Wood sorrel	1700	2800

For the determination of β-carotene, the A.O.A.C. chromatographic method[3] was employed, with the exception that heavy magnesium oxide (British Drug Houses) was used as the adsorbant. Good separation of the β-carotene fraction was achieved without the use of a filter aid. Carotene was determined spectrophotometrically at 436 mμ with a Unicam spectrophotometer, model SP 600.

Results and discussion

The proximate analysis and mode of consumption of the plants studied are shown in Table I. It can be seen that most of the plants are green and leafy in nature and that the leaves and stalks represent the edible portion. For consumption, the leaves and stalks are usually boiled, then drained and mixed with hot olive oil in which onions have been fried. Some of the plants, however, are eaten raw or pickled in vinegar. It should be pointed out that the prepared plant, along with bread, normally constitutes the entire meal, especially in the diets of the lower-income groups. During the winter and spring months, such meals may be consumed 3 or 4 times per week and the intake at a meal may be as high as 150 g. of plant material.

The results in Table I show that the ash values in most instances tended to be high compared with values normally cited for green, leafy plants. Other data from this laboratory[10] have shown that the ash values for most plants grown in Lebanon are higher than those reported in other parts of the world. These findings may be related to the relatively high mineral content of the soils of Lebanon.[11]

The values for ether extract in most of the samples analysed were within the expected range. In the samples of hyssop and old-man's beard, for which the ether extract values were high, the moisture content was relatively low. On a dry-weight basis, therefore, the ether extract levels would be comparable with those of the other plants.

The protein content of most of the plants was about 1–2% of the fresh weight. Some widely used plants such as mallow and amaranth, however, contained as much as 5% protein. Considering the fact that the prepared plant, along with bread, constitutes the entire meal, the essential amino-acid content of these plants may be of considerable significance. Table II shows the essential amino-acid values for 8 of the most frequently consumed plants. When these values were compared with the FAO pattern,[12] lysine was seen to be the most limiting amino-acid. These plants, therefore, could not be considered effective supplements for bread protein, since bread is also low in lysine.

The finding of low lysine values prompted the investigation of the availability of this amino-acid in both raw and prepared plants. Results on 11 commonly eaten plants, presented in Table III, indicate that the lysine was readily available in the raw plants. Cooking and pickling had essentially no effect on the availability of lysine in these plants.

Since green plants are usually considered important sources of vitamin-A precursors, it was of interest to determine the carotene contribution of the edible wild plants to the Lebanese diet. The values in Table IV indicate that most of the plants analysed are excellent sources of carotene. Depending upon the plant eaten, a daily intake of 100 g. edible portion would contribute 1000–10,000 i.u. of dietary provitamin A per day. This contribution is significant especially when the frequency of consumption is considered.

The results obtained establish the importance of edible wild plants in the Lebanese diet. Further work seems justified to study other nutrients and to expand the list of edible plants.

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THE ISOLATION OF LEAF COMPONENTS. II*

By D. THIRKELL and G. R. TRISTRAM

A separation of the lipid fractions is described and identification of various lipid classes is achieved. Individual compounds in these classes are separated and some of them tentatively characterised by R_F value, colour in daylight and in ultra-violet, and absorption maxima.

Introduction

The lipids from lucerne, which include the normal lipid classes and the various pigments which are present, form a complex mixture of compounds. Various analyses have been carried out on plant lipids in order to separate the individual classes, but certain of these procedures, e.g., saponification, characterisation of components (e.g., sterols, fatty acids), give no clue to their origin. It was therefore decided to attempt the separation of the complete mixture without prior treatment.

Booth[1] separated the lipid components of leaves by two-dimensional paper chromatography on Whatman No. 31 filter papers impregnated with zinc carbonate. The solvent systems used were 1% acetone in light petroleum (b.p. 40–60°) followed by methanol/water (23/2 : v/v) over a paraffin-coated section of the paper. The method was not intended as a means of preparation. Hirsch & Ahrens[2] outlined a method for the separation of the lipid classes from plasma and from artificial mixtures of lipids by selective elution procedures from silicic acid columns. Preliminary experiments using the method of Booth and thin-layer chromatography with silica gel G suggested that it might be possible to adapt the method of Hirsch & Ahrens for the separation of at least some of the components present in a solvent extract of green lucerne leaves.

Experimental

Methods and materials

(1) *Source of the leaf lipid/chlorophyll mixture*

The material was supplied by British Glues & Chemicals Ltd. Lucerne was milled in the Impulse machine (Chayen & Ashworth[3]) in an alkaline medium at pH 10. The protein was precipitated at pH 4·5 and the lipid/chlorophyll 'complex' was co-precipitated quantitatively. The protein was freed from chlorophyll, lipid and water by azeotropic drying with hexane ethanol (75/30 : v/v) and the lipid/chlorophyll mixture recovered from the solvent mixture in a yield of 0·6–0·8% of the weight of fresh leaf.

* Part I: *J. Sci. Fd Agric.*, 1961, **12,** 502

(2) *Reagents*

Silicic acid was from the Malinckrodt Company, while light petroleum (b.p. 60–70°), diethyl ether (*d* 0·717) and methanol were all glass-distilled before use.

(3) *Identification of components*

Wax.—Fractions thought to contain wax were dissolved in the minimum amount of warm ether and the wax was precipitated by the addition of 5 volumes of acetone.

Carotenoids.—These were identified by their absorption spectra and by thin-layer chromatography on silica gel G (Merck). The R_F values on the latter were compared with those of known compounds.

Sterols and sterol esters were identified by the Lieberman–Burchardt reaction.[4–6]

Triglycerides were characterised by the method of Feigl & Frehden.[7]

Fatty acids.—These were identified by either reaction to bromocresol green (Hirsch & Ahrens[2]) or by titration of aliquots in neutral ethanol/phenolphthalein with 0·1N-sodium hydroxide.

Chlorophylls were identified by spectrophotometry.

Total phosphorus was estimated by the method of King.[8]

(4) *Column chromatography*

(a) *Column adsorbent*

The chromatographic procedure was based on the method of Hirsch & Ahrens,[2] and after pre-treatment of the silicic acid, a column (17 cm. × 1 cm.) was prepared as described.

(b) *Charging the column*

A solution of 1·6366 g. of material was made in 10 ml. of warm light petroleum (b.p. 60–70°) and 5 ml. (equivalent to 818·3 mg.) were applied to the top of the column which was blacked out with opaque paper to prevent photo-oxidation of pigments. With the refractionation of fraction 8, 362·8 mg. of material, dissolved in light petroleum (b.p. 60–70°) containing 10% diethyl ether, were similarly applied.

(c) *Elution of the column*

The solvent systems are shown in the elution diagrams, and pressure (about 4 p.s.i.) applied to the top of the column gave a satisfactory flow rate. The gradual increase in solvent polarity accounts for the orderly separation achieved.

(d) *Analysis of the fractions*

Fractions of 10 ml. were collected on a syphon-type fraction collector and each fraction was taken to dryness, twice redissolved in 99% ethanol, and taken to dryness again under vacuum. An analysis by weight was carried out and elution diagrams drawn. Each fraction was pooled and dissolved in either light petroleum (b.p. 60–70°) or diethyl ether and stored in the dark. Aliquots of these solutions were taken for identification.

(5) *Thin-layer chromatography*

The plates (20 cm. square) were prepared with the Desaga apparatus [supplied by Camlab (Glass) Ltd.] using silica gel G (according to Stahl). Spots were applied 2 cm. from the base with the specially available 10 μl. pipette. They were developed for 1½–2 h. with hexane/ether (70/30 v/v).

Analysis of the thin-layer plates.—The spots were marked and their visible colours noted and the procedure was then repeated under ultra-violet light. After elution into hexane or ether, absorption spectroscopy was carried out over a wide wavelength range.

Results

Fig. 1 shows the separation which was obtained with the lipid/chlorophyll mixture and the positions where the cuts were made for the individual fractions. Table I shows the analysis from Fig. 1 in terms of weight. A recovery from the column of just over 86% was achieved.

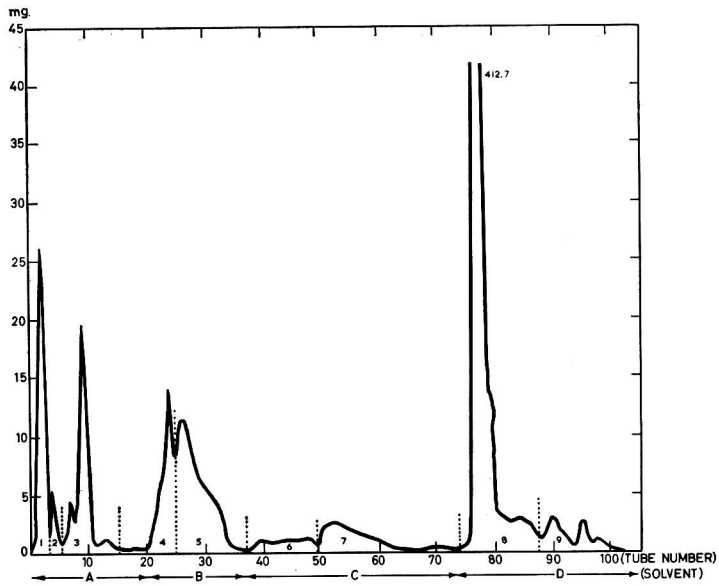


FIG. 1.—*Elution curve of the chlorophyll-lipoprotein fraction from lucerne*

column adsorbent: silicic acid 100 mesh

Elution solvents: A 60–70° light petroleum with 1% diethyl ether
B " " " " 4% " "
C " " " " 8% " "
D Methanol

During the evaporation of each collector fraction, a solvent trap was incorporated. The contents of this accounted for a further 9% and it is assumed that the remainder was left on the column. After elution, the column looked slightly green at the top and this material was probably of a protein nature and present in the material as a result of the method of preparation.

As fraction 8 contained 61% by weight of the original material, this was refractionated on a similar column with a slower increase in solvent polarity. The fractionation was again carried out by weight analysis and the elution pattern is shown in Fig. 2. The distribution of weight throughout these fractions is shown in Table II.

Separation of the different fractions was done by thin-layer chromatography and the results are shown diagrammatically in Figs. 3–4. Fig. 3 shows the components isolated from fractions 1–9 (excluding fraction 8), and Figs. 4 (*a*) and (*b*) show the components of the sub-fractions from fraction 8.

The analytical results obtained from all of the fractions of the original material are summarised in Tables III–V.

Comparison of the different sub-fractions was done by at least three similarities out of visible colour, colour under ultra-violet, R_F value on the plate, and where determined, absorption spectra. It is seen that 37 separate compounds were isolated in the sub-fractions of fraction 8.

Discussion

The method appears to give a good degree of initial separation of the complex lipid mixture into most of the lipid classes which are known to be present. It also gives a reasonable method

Table I

Analysis of chlorophyll–lipoprotein fraction from lucerne

(Analyses by weight)

Fraction	Eluting solvent	Wt. of fraction, mg.	% of wt. applied to column
1	60–70° Light petroleum + 1% of diethyl ether	33·1	4·0
2	,, ,, ,, ,, ,, ,, ,, ,,	7·8	11·0
3	,, ,, ,, ,, ,, ,, ,, ,,	34·6	4·2
4	60–70° Light petroleum + 4% of diethyl ether	36·3	4·4
5	,, ,, ,, ,, ,, ,, ,, ,,	50·3	6·1
6	60–70° Light petroleum + 8% of diethyl ether	9·6	1·2
7	,, ,, ,, ,, ,, ,, ,, ,,	21·0	2·6
8	Methanol	499·9	61·1
9	,,	12·8	1·6
Total wt. and % recovered		705·4	86·2
Recovered from vacuum pump		73·9	9·0
Presumably remaining on column		39·0	4·8
Wt. of material applied to column		818·3	100·0

for the quantitative preparation of these classes free from one another in most cases and from which further investigation into the members of each class can be carried out.

The overlap of fractions 4 and 5 could probably be overcome by either a slower increase in solvent polarity or by lengthening the column.

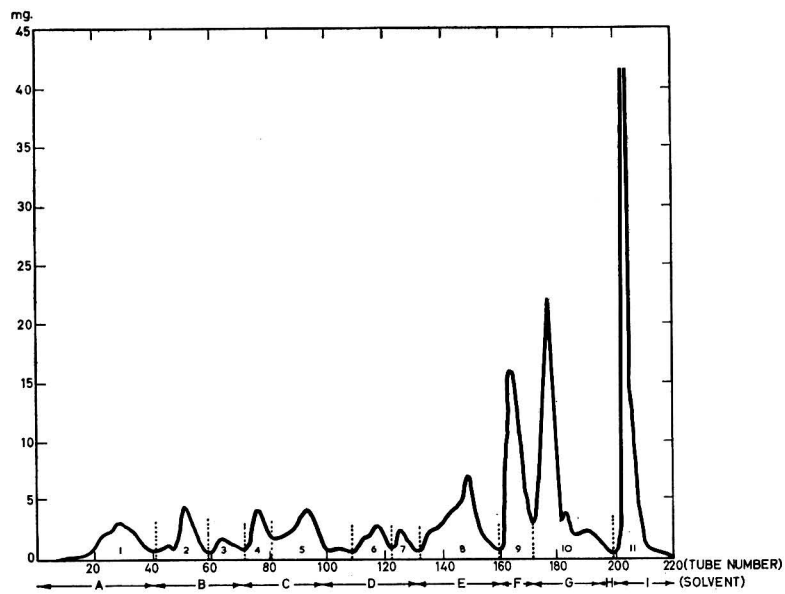


FIG. 2.—*Refractionation of fraction* 8 *in Fig.* 1

Elution solvent: 60–70° light petroleum with (A) 10%, (B) 15%, (C) 20%, (D) 25%, (E) 32·5%, (F) 50%, (G) 75% diethyl ether; (H) diethyl ether; (I) methanol

Table II

Weight analysis of the sub-fractions of fraction 8

Fraction	Wt., mg.	% of original	Eluting solvent
8–1	21·4	5·9	60–70° Light petroleum + 10% of diethyl ether
–2	14·3	3·9	,, ,, ,, ,, 15% ,, ,, ,,
–3	5·8	1·6	,, ,, ,, ,, 15% ,, ,, ,,
–4	13·7	3·8	,, ,, ,, ,, 20% ,, ,, ,,
–5	23·6	6·5	,, ,, ,, ,, 20–25% ,, ,, ,,
–6	9·5	2·6	,, ,, ,, ,, 25% ,, ,, ,,
–7	5·2	1·4	,, ,, ,, ,, 25% ,, ,, ,,
–8	37·9	10·5	,, ,, ,, ,, 32·5% ,, ,, ,,
–9	49·8	13·7	,, ,, ,, ,, 50% ,, ,, ,,
–10	69·5	19·2	,, ,, ,, ,, 75–100% ,, ,, ,,
–11	83·8	23·1	Methanol
Total :	334·5 mg.	92·2%	

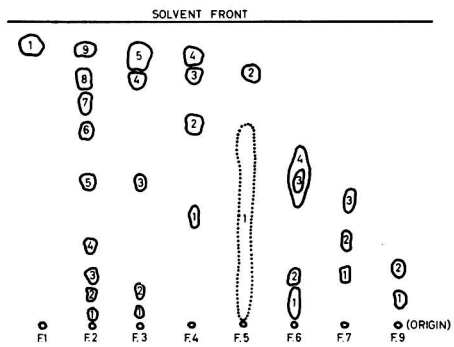


FIG. 3.—*Chromatographic spots of components from fraction (F)* 1–7 *and* 9

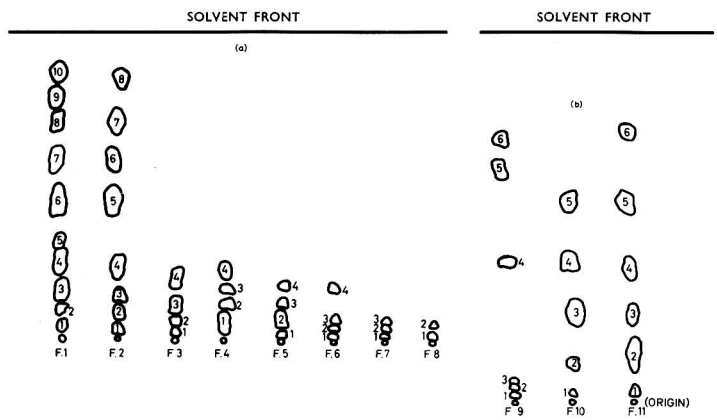
By refractionation of fraction 8, which contained 61% of the original material, with a slower increase in solvent polarity, it was shown to contain a wide variety of components.

It would appear that, providing that the column is long enough and that the material is applied to the column in a concentrated form, a large amount of material can be separated without overloading the column. However, the separation of fairly large quantities would appear to be essential if sufficient of the smaller fractions is to be available for complete characterisation of these fractions.

The use of thin-layer chromatography has shown that each of the fractions separated contains anywhere between 1 and 10 compounds. The colours which were visible under ultra-violet were many and varied and consequently it was difficult to name each one accurately. Because of the small amounts of each compound isolated in each spot on the plate, characterisation of most of these compounds has not been possible. In some cases where absorption spectra were carried out, a mixture of compounds, even in one spot, was indicated. From the number of green spots it would appear that many degradation products of chlorophyll are present and, again, this may be anticipated from the method of preparation of the original material.

In some cases, compounds appear to be similar although running with slightly different R_F values. This may depend upon the number and type of components running together in each fraction.

The techniques quoted do give a means of separation of leaf lipids without any pre-treatment



FIGS. 4*a and* 4*b*.—*Chromatographic spots of components of sub-fractions* (*F*) *from fraction* 8

Table III

Analytical data on fractions 1–9

Spot	Colour	Colour under ultra-violet	R_F on plate	$\lambda_{max.}$	Identity	Comment
Fraction 1						
—	Colourless	Purple	0·916	—	Wax	—
Fraction 2						
—	Orange	—	—	—	Carotene	Absorption spectra
1	Colourless	Blue/violet	0·032	—	—	—
2	,,	,,	0·104	—	—	—
3	,,	,,	0·161	—	—	—
4	,,	,,	0·258	—	—	
5	,,	,,	0·464	—	—	—
6	,,	Violet	0·636	—	—	—
7	,,	Blue/violet	0·723	244, 254, 262, 282	?	$\lambda_{max.}$ in hexane
8	,,	Almost white	0·810	—	—	—
9	Yellow/orange	Yellow/green	0·904	427, 447, 476	Carotene	$\lambda_{max.}$ in hexane
Fraction 3						
—	Yellow	—	—	—	Carotene	Absorption spectra
					Sterol esters	30–40 min. reaction
1	Colourless	Violet	0·045	—	—	—
2	,,	,,	0·109	—	—	—
3	Yellow	Green	0·464	427, 444, 472	Carotene	Absorption spectrum in hexane
4	Colourless	Blue/violet	0·807	—	—	—
5	,,	Purple	0·871	—	—	—
Fraction 4						
—	Pale green	—	—	—	Fatty acids	No change with bromocresol green : positive by titration
				—	Triglyceride	Production of acrolein
1	Pale green	Pink	0·348	—	—	—
2	Colourless	Violet	0·640	—	—	—
3	,,	,,	0·807	—	—	—
4	,,	Purple	0·871			
Fraction 5						
—	Pale green	—	—	—	Fatty acids	—
1	Colourless	Light purple streak	—	—	—	—
2	,,	Almost white	0·815	—	—	—
Fraction 6						
—	Yellow/green	—	—	—	Sterols (Δ^7, $\Delta^{5,7}$) Pigments	Immediate reaction
1	Colourless	Pink/purple	0·080	410, 610, 670	Chlorophyll-type pigment	Absorption spectrum in 60–70° light petroleum
2	,,	Red/purple	0·155	—	—	—
3	Yellow/green	Green	0·464	426, 441, 471	Xanthophyll	Absorption in 60–70° light petroleum
4	Colourless	Pink	0·477	—	—	—
Fraction 7						
—	Yellow/green	—	—	—	Sterols (Δ^5) Pigments	10 min. reaction time
1	Pale green	Pink	0·168	410, 615, 662	Chlorophyll-type pigment	Absorption in 60–70° light petroleum
2	Pale green	Pink	0·278	—	—	—
3	Yellow	Green	0·400	414, 440, 470	Carotenol	Absorption in 60–70° light petroleum

Table IV

Analytical data on fraction 8

Fraction	Spot	Colour	Colour under ultra-violet	R_F on plate	$\lambda_{max.}$	Identity	Comment
8	—	Dark green	—	—	—	Mixture	61% of the original
8 (1)	1	Pale green	Red	0·039	—	—	—
	2	,, ,,	Red/brown	0·098	430, 472, 615, 662·5	Chlorophyll a*	Absorption in ether
	3	Green	Pink	0·141	—	—	—
	4	Pale green	,,	0·219	—	—	—
	5	,, ,,	,,	0·281	—	—	—
	6	Colourless	Green	0·395	414, 440, 470	Carotenol	Absorption in 60–70° light petroleum
	7	,,	Purple	0·514	—	—	—
	8	,,	Green	0·628	Indistinct triple-peaks	Carotenoid ?	Absorption in 60–70° light petroleum
	9	,,	Purple	0·685	—	—	—
8 (2)	1	Green	Red/brown	0·028	—	—	—
	2	Dark green	Black	0·079	430, 472, 615, 662·5	Chlorophyll a*	Absorption in ether
	3	Pale green	Red	0·129	—	—	—
	4	Colourless	Purple	0·203	—	—	—
	5	,,	Green	0·395	424, 440, 470	Carotenol	Absorption in 60–70° light petroleum
	6	,,	Purple	0·514	—	—	As fraction (1) 7
	7	,,	,,	0·628	—	—	As fraction (1) 8
	8	,,	Blue/purple	0·755	—	—	—
8 (3)	1	Green	Red	0·021	—	—	—
	2	Pale green	Dark red	0·056	—	—	—
	3	,, ,,	Pink	0·098	—	—	—
	4	Colourless	Purple	0·179	—	—	—
8 (4)	1	Pale green	Pink	0·047	—	—	—
	2	Dark green	Red	0·099	—	—	As fraction (3) 3
	3	Pale green	Pink	0·149	—	—	—
	4	Colourless	Purple	0·203	—	—	As fraction (2) 4
8 (5)	1	Dark green	Red	0·018	—	—	—
	2	Pale green	Pink	0·056	—	—	As fraction (3) 2
	3	Colourless	,,	0·109	—	—	—
	4	,,	,,	0·160	—	—	—
8 (6)	1	Green	Red	0·017	—	—	As fraction (5) 1
	2	Pale green	Pink	0·039	—	—	As fraction (1) 1
	3	Colourless	Pink/purple	0·065	—	—	—
	4	,,	,,	0·160	—	—	As fraction (5) 4
8 (7)	1	Green	Red	0·021	455, 510, 640, 660	*Chlorophyll b (?)	Absorption in diethyl ether
	2	Pale green	Pink	0·039	—	—	As fraction (1) 1
	3	Colourless	Pink/purple	0·065	—	—	As fraction (6) 3
8 (8)	1	Green	Red	0·022	—	—	As fraction (7) 1
	2	Dark green	,,	0·051	—	—	—
8 (9)	1	Green	Pink	0·032	—	—	—
	2	Colourless	Blue	0·054	—	—	—
	3	,,	Pink	0·072	—	—	—
	4	,,	Purple/green	0·473	—	—	—
	5	,,	Blue	0·784	—	—	—
	6	,,	Light blue	0·890	257, 262, 271	Quinone (?)	Absorption in 60–70° light petroleum
8 (10)	1	Pale green	Pink	0·032	455, 510, 640, 660	*Chlorophyll b (?)	Absorption in diethyl ether
	2	Colourless	Purple	0·131	—	—	—
	3	,,	Purple/green	0·301	—	—	—
	4	,,	,,	0·473	—	—	As fraction (9) 4
	5	,,	,,	0·685	—	—	As fraction (1) 9 (?)
	6	,,	Light blue	0·902	257, 262, 271	Quinone (?)	As fraction (9) 6 (?)
8 (11)	1	Pale green	Pink	0·032	455, 510, 640, 660	*Chlorophyll b (?)	Absorption in diethyl ether
	2	Colourless	Violet	0·151	—	—	—
	3	,,	Purple/green	0·301	—	—	As fraction (10) 3
	4	,,	,,	0·450	—	—	—
	5	,,	,,	0·685	—	—	As fraction (1) 9 (?); fraction (10) 5
	6	,,	Light blue	0·908	257, 262, 271	Quinone (?)	As fraction (9) 6 (?)

* Comparison with figure quoted by Mackinney[9]

Table V

Analytical data on Fraction 9

Spot	Colour	Colour under ultra-violet	R_F on plate	$\lambda_{max.}$	Identity	Comment
—	Pale green	—	—	—	Pigment : phospholipid	Colour : trace of P present
1	,, ,,	Pink/purple	0·082	—	Phospholipid (?)	Trace of P present
2	,, ,,	Pink	0·187	412, 660	—	—

(e.g., saponification), but a method is now required which will allow the separation of all the compounds in each fraction, in sufficient amounts to permit complete characterisation to be carried out. The compounds which are named are identified tentatively on the evidence at present available, and more definite proof should be forthcoming with the separation of larger quantities of each compound.

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BUFFERING CAPACITY OF SWEET SORGHUM: THE EFFECTS OF NITROGEN CONTENT, GROWTH STAGE AND ENSILAGE

By M. J. PLAYNE*

The buffering capacities of sweet sorghum plant material at three stages of growth and at two plant nitrogen levels were compared before and after 5 days of ensilage.

Buffering capacity decreased with plant age, but showed no significant relation to total nitrogen content or non-protein nitrogen content of the plant. The buffering capacity of the fermented forage was, overall, about twice that of the unfermented forage, but this increase was not apparently related to the increase in the products of protein breakdown which occurred during ensilage.

Introduction

Many plants have pH values about 6, and good-quality silages, made from unwilted material, usually have values about 4. From consideration of pK_a values, the main plant buffers within this pH range are the organic acids and their salts. The amino-acids mostly possess pK_a values which lie outside the range of pH 6-4. However, decarboxylation of amino-acids with the production of basic residues may also occur. These bases may neutralise lactic acid formed.[1] Although many workers[2–5] have inferred that nitrogenous compounds cause buffering in plant material, there is little experimental evidence to support this suggestion.

In the present experiment, the relation was examined between the buffering capacity of sweet sorghum (*Sorghum vulgare* var. Saccaline) and the total nitrogen content and non-protein

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nitrogen (NPN) content of the forage before and after 5 days of ensilage. The plant was grown under two nitrogen fertiliser regimes and was sampled at three stages of growth.

Experimental

(a) *Cultural procedure*

Sweet sorghum plants were grown in the field to a double split-plot design with four replications. No fertiliser was applied to half of the plots (N_0) ; to the remaining plots (N_1), a total of 194 lb. of N per acre was applied as sulphate of ammonia in three applications over the growing period. The crop was grown in the summer season (planted on October 19, 1961) and was irrigated when necessary.

(b) *Sampling procedure*

The crop was sampled at 6, 9 and 12 weeks after planting, that is, at a pre-booting stage, at an early flowering stage, and at a soft dough stage. Sampling was always carried out between 10.30 a.m. and 1.00 p.m. to reduce the possibility of variation from diurnal fluctuations. The samples were chopped to about $\frac{1}{4}$ in. lengths. Subsampling was carried out using the ' quartering ' method.

Silage was made in miniature laboratory silos of one kilogram green matter capacity as used by Catchpoole.[6]

(c) *Analytical procedure*

So that weights of fresh plant material containing close to 1·0 g. of dry matter could be used in the electrometric titration of buffer capacity, samples for estimation of dry matter were taken on the day before the actual sampling day. Where differences occurred between these determinations of dry matter content and those made at the actual sampling time, the buffer curves were corrected to be representative of material containing 1·0 g. of dry matter.

Determination of dry matter.—Duplicate chopped samples each of 200 g. of fresh weight were dried to constant weight in a forced-draught oven at 75° for about 48 h.

In the determination of buffering capacity of 5-day-old silage, fresh silage samples for determination of buffering capacity were stored at −15° until the content of dry matter had been determined on another sample by the method described for plant material. It was realised that volatile substances in the silage might be lost by this method,[7] but it was considered that the error which could occur would be insignificant in view of the large increases in buffering capacity and in NPN which were likely during ensilage.

Total nitrogen.—Determinations were made on 0·4 g. of dried, ground material. The Kjeldahl method of McKenzie & Wallace[8] was modified to suit this quantity of material.

Non-protein nitrogen.—Twenty-five g. of fresh plant material was macerated and then repeatedly extracted with 80% v/v ethanol. The total nitrogen content of an aliquot of the extract was determined after removal of the ethanol from the extract by evaporation under reduced pressure at 40°.

Buffering capacity.—Buffering capacity over the range of pH 6 to pH 4 was determined by electrometric titration with a Cambridge bench pH meter. A fresh sample containing 1·0 g. of dry matter was macerated in 200 ml. of water. With continuous stirring, a pH reading of the suspension was made (initial pH). 0·2N-Sodium hydroxide was added slowly to the suspension to pH 7. After equilibrium had been reached, 0·2N-hydrochloric acid was added in successive volumes of 0·5 ml., and pH was plotted against volume of standard acid to pH 3.

Results and discussion

At the pre-booting stage (age 1), the buffering capacity of the plant material between pH 6 and 4 was about twice that at the later growth stages (the flowering stage, age 2, and the soft dough stage, age 3). The total nitrogen content of the dry matter decreased significantly ($P < 0.05$) throughout all three growth stages as shown in Table I. The high buffering capacity of silage made from immature sweet sorghum may help explain why poor-quality silage of high pH is often produced from such material.

There was no significant relation between buffering capacity and total nitrogen content of

Table I

Effects of plant growth stage, nitrogen fertilisation, and ensilage on buffering capacity

Treatment		Plant (A)				Silage (B)			Difference (B − A)	
		Initial pH	Buffer capacity, mequiv.*	Total N, %†	Non-protein N, %	Initial pH	Buffer capacity, mequiv.	Non-protein N, %	Buffer capacity, mequiv.	Non-protein N, %
Age 1	N_0	5·70a‡	43·0a	1·68	0·30ab	4·25e	72·8e	0·52de	29·8e	0·22e
	N_1	5·89a	38·8a	2·26	0·32ab	4·17e	73·6e	0·66e	34·9e	0·34e
Age 2	N_0	5·83a	20·2b	0·86a	0·12c	3·95f	58·8f	0·42ad	38·6e	0·30e
	N_1	5·92a	17·6b	1·19	0·39ad	3·82f	59·6f	0·62e	42·0e	0·23e
Age 3	N_0	5·87a	18·6b	0·46	0·10c	3·84f	53·2f	0·20bc	34·6e	0·10e
	N_1	5·87a	20·4b	0·95a	0·22bc	3·72f	58·6f	0·40ad	38·0e	0·18e

* Milli-equivalents of acid required to reduce the pH of 100 g. dry matter from 6 to 4.
† Percentage of oven-dried material.
‡ Values for a particular determination which do not differ at $P < 0.05$ are marked by a common small letter. All values are means of 4 replications. Statistical significance was determined by Duncan's multiple range test as least significant values are not suitable here. The correlation coefficient calculated for individual values of B − A was 0·09.

sorghum plant material. This was most clearly demonstrated in the comparison of material from unfertilised (N_0) and fertilised (N_1) plots. Fertilisation increased the nitrogen content of the plant material at all stages of growth, but the buffering capacity of the plant was little affected by fertilisation (Table I).

After 5 days of ensilage, the buffering capacity of fresh silage was markedly higher than that of the fresh plant material (Table I). Over the three sampling ages, the buffering capacity of the silage was about twice that of the plant material. This observation agrees with the results of other workers on different plant species.[2, 5]

Swedish workers[2, 3] have suggested that the buffering capacity of silage is higher than that of the plant material because of increased amounts of the products of protein breakdown in the silage. However, when the increases in the percentage of NPN of the dry matter which occurred with ensilage are compared with the increases in buffer capacity for the various treatments, it is apparent that there was no clear relationship (Table I). Although increases always occur in the mean values of both NPN and buffering capacity on ensilage of plants, regression analysis showed that there was no definite relationship ($r = 0.09$).

The addition of fertiliser to the N_1 sample plots has probably affected factors such as cation content, organic acid content and sugar content, as well as nitrogen content of the plant. Changes in the content of such constituents may well have masked the effects of increased nitrogen content on the buffering capacity of the plant. However, comparison of the silage initial pH values of the two fertiliser treatments suggests that acid formation was not adversely affected by high nitrogen fertilisation.

It should be remembered, too, that the levels of nitrogen in sweet sorghum are low compared with those in many other plants, especially legumes, even when fertiliser is added to the soil. It may be that buffer capacity is affected to a degree by nitrogenous compounds, although there is no reason to expect much buffering due to amino-acids in the pH range of 6 to 4. The examination of one species can only give an indication of what might be common to many forages. Nevertheless, this work has suggested that the contribution of the nitrogenous compounds to the buffering capacity of the plant may not be large.

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STUDIES ON PROTEIN HYDROLYSIS. VII.*—Anaphylaxis in Guinea Pigs Produced by some Fractions of Antigenic Casein Hydrolysates†

By B. E. BAKER and E. I. BERTOK

The removal of the stable foam from an antigenic enzymic casein hydrolysate solution, reduced the anaphylactoid reaction produced by the hydrolysate and also reduced its antigenicity. Solutions of freeze-dried foam fractions were in most instances distinctly antigenic, they cross-reacted with milk proteins and produced strong anaphylactoid shock in guinea pigs.

Introduction

In the course of investigations[1, 2] carried out in this laboratory, on the anaphylactoid and anaphylactic shock produced in guinea pigs by enzymic casein hydrolysates, it has been shown that hydrolysates which produce stable foams tend to be antigenic. It was suggested that the foaming of the hydrolysate was due to the presence therein of proteins or large protein fragments which could cross-react with the original product.

The purpose of the study now reported was to examine the possibility of removing the antigenic constituents from an antigenic hydrolysate and to determine the nature of these constituents.

Experimental

Materials and methods

Casein hydrolysates.—These were commercial spray-dried enzymic casein hydrolysates. The samples designated as hydrolysate A_1, A_2 etc., were taken from different batches of the same commercial product.

Casein and whey.—Casein was prepared from a composite sample of milk (Macdonald College herd) according to the method of Warner.[3] The casein was freeze-dried and stored at 5° until ready for use.

Isolation of foam fraction.—Spray-dried enzymic casein hydrolysate (300 g.) was dissolved in physiological saline or in water, and the volume adjusted to 3000 ml. with physiological saline or with water. An aliquot (300 ml.) was transferred to a separatory funnel (500 ml.) and then shaken vigorously for about 2 min. The solution was set aside for 2 min. and then the lower liquid fraction (R_1) was separated from the upper stable foam (F_1).

One hundred ml. of fraction R_1 were transferred to a separatory funnel (100 ml.) and a fraction R_2 was obtained in the same manner as was R_1. Similar fractions R_3 and R_4 were prepared. The corresponding foam fractions were discarded.

Animal maintenance was as described in a previous paper,[1] as were tests for anaphylactoid and anaphylactic shock, measurement of foam formation and of transmittance.

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Anaphylactoid shock produced by hydrolysate fractions

Three enzymic casein hydrolysates (A_1, A_2, A_3) which displayed high foam breaking times[2] were fractionated by the foaming technique described above. Fractions F_1, R_4 and a solution of the hydrolysate before fractionation were freeze-dried (Virtis Laboratory Freeze Drier). Samples (0·25 g.) of the freeze-dried product were dissolved in physiological saline and the volumes were adjusted to 10 ml. Guinea pigs (250–300 g.) were divided into four groups of five animals each (20 in all). All four animals of a given group each received an intraperitoneal injection (2 ml. of a 10% solution) of one of the above solutions. Table I shows the foam-breaking times and the transmittances of the three fractions (solutions of the freeze-dried materials) and the anaphylactoid responses produced by the intraperitoneal injections. It will be noted that the R_4 fractions all gave lower foam breaking times and higher transmittances

Table I

Anaphylactoid shock (in vivo) *in guinea pigs produced by hydrolysate fractions*

Hydrolysate no.	Solvent used in fractionation	Fraction	Foam breaking time of solution of freeze-dried fraction, sec.	Transmittance at 515 mμ	Anaphylactoid shock Animal no. 1	2	3	4	5
A_1	Water	O	88	64	+	+	++	+	
		R_4	26	89	–	–	–	–	–
		F_1	>10,000	62	++	++	+	++	++
A_2	Water	O	180	72	++	++	+	++	
		R_4	48	74	+	+	+	+	+
		F_1	>10,000	41	++	++	++	+++	++
A_3	Saline	O	>5000	84	+	++	++	+	
		R_4	180	85	++	+	+	+	+
		F_1	>5000	47	+++	+++	++	++	++
A_3	Water	O	>5000	84	++	++	++	+	
		R_4	85	85	–	–	–	–	–
		F_1	>5000	47	+++	++	+	+++	++

than did the corresponding original hydrolysates (Fraction O). The removal of the F_1 fraction from the hydrolysates led to a decrease in the anaphylactoid shock produced by the hydrolysates.

Anaphylactic shock produced by hydrolysate fractions

Four enzymic casein hydrolysates (A_3, A_4, A_5, A_6) were dissolved in physiological saline and two hydrolysates (A_7, A_3) were dissolved in distilled water. The solutions were shaken to produce the stable foam. The two fractions F_1 and R_4 were then separated and then freeze-dried. Samples (0·25 g.) of the freeze-dried products as well as of the original hydrolysate (Fraction O) were dissolved in physiological saline and the volumes adjusted to 10 ml. The antigenicity of each fraction and the capacity of each fraction to cross-react with milk proteins were checked by animal tests as follows. Guinea pigs (250–300 g.) were sensitised by three intraperitoneal injections (2·0 ml., 2·5 ml., 2·5 ml.) of reconstituted skimmed milk (10 g. skimmed milk powder/100 ml. of physiological saline) or solutions of the fractions, at 3-day intervals. Twenty-one days after the last sensitising injection, each animal received an intravenous injection of 1 ml. of reconstituted skimmed milk or a solution of a particular fraction. Table II shows that all the foam fractions (F_1) tested were more antigenic than the corresponding residue (R_4) fractions. The tendency of the F_1 fraction to cross-react with milk proteins was consistent with the hypothesis that the substances which were isolated from the hydrolysate by the foaming technique were unhydrolysed milk proteins or protein fragments.

Discussion

Previous experiments[1] showed that hydrolysates with low transmittances are more likely to produce anaphylactoid shock in guinea pigs than those with high transmittances. It was suggested that the products of the browning reaction may play an important rôle in anaphylactoid shock produced by casein and casein degradation products subjected to severe heat

Table II

Anaphylactic shock (in vivo) *in guinea pigs produced by hydrolysate fractions*

Hydrolysate no.	Solvent used in fractionation	Fraction	Foam breaking time of solution of freeze-dried fraction, sec.	Sensitising material	Challenging material	Challenging conditions Animal no. 1	2	3	4
A_4	Saline	O	>10,000	R_4	R_4	+++	++	−	
				F_1	F_1	+++	++++	++++	
				Skimmed milk	R_4	+	++		
		R_4	360	,, ,,	F_1	++	++	+++	+++
				R_4	Skimmed milk	++	+++	+	
		F_1	>50,000	F_1	,, ,,	++	++++	++++	
A_5	Saline	O	>2000	R_4	Skimmed milk	+	+	+	
			210	F_1	,, ,,	+	+	++	++
		R_4		Skimmed milk	R_4	++[a]	+		
		F_1	>5000	,, ,,	F_1	++++	++++	++	
A_6	Saline	O	>1000	R_4	Skimmed milk	++	+		
				F_1	,, ,,	+++	++++		
		R_4	360	Skimmed milk	R_4	−	+		
		F_1	>50,000	,, ,,	F_1	+++	+++		
A_7	Water	O (72)[b]	180	O	Skimmed milk	++	++	+	++
		R_4(74)[b]	48	R_4	,, ,,	+	−	+	−
		F_1(41)[b]	>10,000	F_1	,, ,,	++	++	++++	+++
A_8	Saline	O (84)[b]	>5000	O	Skimmed milk	++	++	+	
		R_4(85)[b]	180	R_4	,, ,,	−	++	+	
		F_1(47)[b]	>5000	F_1	,, ,,	++	++	++	+++
A_3	Water	O (84)[b]	>5000	O	Skimmed milk	++	+	+	
		R_4(85)[b]	85	R_4	,, ,,	++	+	−	−
		F_1(47)[b]	>5000	F_1	,, ,,	+++	+++	++	+++

[a] Two hours after the R_4 injection the animal showed a '4 plus' reaction when challenged with skimmed milk
[b] Figures in brackets are transmittances at 515 mμ

treatment. Later experiments showed that enzymic casein hydrolysates which produce stable foam tend to be antigenic.[2]

In the present work an attempt has been made to remove antigenic substances from antigenic hydrolysates and at the same time to gain some knowledge as to the constitution of these substances. The method employed involved the isolation of the stable foam which forms on solutions of antigenic hydrolysates when they are agitated violently. It was observed that the removal of the stable foam from an hydrolysate reduced the anaphylactoid reaction produced by the remaining hydrolysate (R_4). The removal appeared to be more complete when water was used as a solvent than when saline was used. Solutions of the freeze-dried foam fraction produced extremely stable foam and the hydrolysate minus the foam fraction gave less stable foams. Solutions (10% of freeze-dried material) of the foam fractions had lower transmittances than had the hydrolysate before fractionation (O) or the hydrolysate after the removal of the foam (R_4). The solutions of the foam fractions were distinctly milky in appearance. Consequently the low transmittances of these solutions are almost certainly attributable to their heterogeneous nature rather than to simple optical absorption. It may be concluded from this experiment that the undigested protein or large protein fragments produce anaphylactoid shock in guinea pigs. Previous experiments[1] showed that the enzyme preparation (fresh minced pancreas) employed in the preparation of the hydrolysate gives a strong anaphylactoid reaction and that milk proteins give no anaphylactoid reaction when injected intravenously or intraperitoneally into guinea pigs. It might be suspected, therefore, that the substance or substances in the foam fraction responsible for the anaphylactoid shock are associated with the enzyme preparation.

When interpreting the results of the tests for antigenicity of the hydrolysates and hydrolysate fractions, one should bear in mind that these materials produce anaphylactoid reactions. The results reported in Table I show that these reactions may be as great as +++. In the

experiments where skimmed milk was used for the challenging injections, the reactions were probably due mainly to the antigenicity of the fractions, because skimmed milk does not normally produce an anaphylactoid reaction. The results for hydrolysates A_4–A_7 show that the F_1 fractions were antigenic and that they cross-reacted with milk proteins. The antigenicity of the corresponding R_4 fraction was not apparent from the results obtained when R_4 fractions were used as the challenging material. Although the foam breaking time of the R_4 fractions of hydrolysate A_3 as prepared by the use of water was much less than of the same hydrolysate as prepared with saline, both materials showed about the same tendency to cross-react with milk proteins. It is likely, therefore, that the antigenicity of the hydrolysates is due to the presence therein of unhydrolysed milk proteins or protein fragments capable of cross-reacting with milk proteins. Preliminary electrophoretic tests showed that two constituents of this material have a mobility similar to those of α- and γ-casein, but the more precise identification of these requires much further work.

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STUDIES IN BEEF QUALITY. I.—Development of a System for Assessing Palatability

By J. M. HARRIES, K. BRYCE JONES, T. W. HOUSTON* and JEAN ROBERTSON

A description is given of the arrangement of a tasting panel for assessing the various properties of roast and stewed beef, and of the standardisation of the cooking procedure etc. Some results are given to illustrate the effects of some of the variables.

Introduction

In the United Kingdom, beef quality is generally assessed by visual appraisal of carcass conformation and composition, but there is little scientific basis for predicting the eating characteristics of the meat from its appearance and feel in the raw state. There is growing evidence from the United States that there may be little relationship between commercial grade and palatability. There is no fully documented account of work done in the United Kingdom on the palatability of beef although American literature includes several papers on this subject. In 1949 the Scientific Adviser's Division of the Ministry of Food, and the Low Temperature Research Station of the Department of Scientific & Industrial Research, initiated

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a co-operative investigation of the factors influencing ' drip ' and palatability of frozen beef. A period of one year was spent in developing and testing a system of palatability assessment suitable for this purpose, using staff of the Low Temperature Research Station and of the Scientific Adviser's Division as members of a tasting panel. Since then, the same methods, with minor modifications, have been used by the Food Science & Atomic Energy Division of the Ministry of Agriculture, Fisheries & Food in other studies of beef quality. This work has not been published, but some of the results have been used, e.g. by Law.[1] The present paper describes the evolution of the methods that have become standard in this Division since 1955, and the more important of the methodological problems that arose during the course of our investigation.

Studies of the palatability of other foodstuffs had shown that, provided specified basic rules were applied, and certain limitations accepted, eating quality can be assessed by a panel of selected, trained judges with results which satisfy certain statistical criteria.

These basic rules and limitations are now well established and have been described elsewhere.[2, 3] Briefly, selected and trained judges are asked to assign scores, independently of each other and avoiding the expression of personal preferences, according to predetermined arbitrary scales, to the separate quality characteristics of several samples.

Selection and training of a panel

Selection of judges was carried out as a continuing process concurrently with other tests, the basis of selection being acuity of discrimination between different samples of beef, and consistency of scoring of duplicates. A method frequently used by other workers for selecting tasters is to test discrimination with series of chemical solutions, usually representing the four basic tastes (salt, sweet, sour, and bitter). We prefer to use the foodstuff being investigated because, by so doing, sensitivity to olfactory stimulation (which contributes very greatly to flavour as normally understood) is included, as well as other characteristics of the food.

The purposes of training the panel were to familiarise the members with the procedure of eating and scoring ungarnished and unaccompanied beef; to ensure that panel members interpreted the terms on the score sheet in the same way, in order to avoid the possibility of variation in scores arising from ambiguity of meaning; and to eliminate preference judgments which are likely to be variable from person to person. It was necessary to discover by experiment the best way of cooking and serving the meat so that the panel might reach a satisfactory standard of agreement and discrimination. During the preliminary tests the composition of the panel was changed by progressive selection of the most discriminating and most consistent tasters. Over the last 12 years, many of the original members have left, and been replaced by others. When the co-operative investigation came to an end in 1951 the staff of the Low Temperature Research Station ceased to serve as members of the panel. The number of trained panel members has varied over the years from 8 to 10, but of these the number present at any one panel session has usually been from 6 to 8. Most are laboratory workers, but staff engaged on meat work are deliberately excluded. The members of the panel know nothing of the purpose or design of any particular investigation until they read the final report. They are too few and too expert to be considered representative of the consuming public.

The scoring system

The assessment of palatability characteristics can only be by the recording of sensory experiences,* which is difficult even when a complete vocabulary is available for their description. The investigator who tries to get a number of other people to quantify their (separate) sensations, in order to discover facts about the stimuli he has presented to them, must provide a system of quantification which assists the difficult process of recording, whilst limiting the responses to what is relevant. The score scales now used for roast and stewed beef are shown in Tables I and II. The scales are necessarily arbitrary, and were finalised after a great deal of preliminary

* There are other methods of measuring some of the known components of palatability, notably mechanical means of examining physical properties of texture. The relationship between these measurements and what happens in the mouth during chewing is not, however, very clear, and such methods cannot yet be used as the sole assessment of textural aspects of palatability.

testing, taking into account the tasters' own views concerning ease in use and clarity of meaning. Fat is scored only for off-flavour. Lean is scored for meat flavour, off-flavour, tenderness, juiciness, and the degree to which it has been cooked. The last assessment was found to be necessary because in spite of rigid temperature control of the cooking process occasionally a joint of meat would still be red when removed from the oven. Other workers have had the same experience (e.g.,[4]), but as far as we are aware no way of eliminating this phenomenon has been established. The panel members are asked to score all the samples for ' degree of doneness '* in the hope that collection of such data may ultimately enable this phenomenon to be linked to some production variable.

It will be seen from Table I that the mid-point of the scale for ' degree of doneness ' is labelled ' neither overdone nor underdone ' and tasters have it impressed upon them that this does not in fact represent the degree to which they would prefer to have their meat cooked. What is required is a judgment, based on colour and texture, of whether a sample is overdone, underdone or neither.

The scales for juiciness and tenderness have no mid-point at zero. Such a mid-point would indicate a lack of sensation for these characteristics, which is impossible. This gap in the continuum of the scale provides some difficulty in the analysis of the subsequent results, which is overcome by subtracting half a mark from each positive score given, whether for juiciness or for tenderness, and adding half a (positive) mark to each negative score given. It is debatable how far this adjustment is necessary or desirable. Since these scales are arbitrary, and not expressed in terms of any known unit of measurement, we do not know by how much the steps between successive scores may vary. At the time this system was developed there was considerable discussion (e.g.,[5]) of the importance of this possible inequality, and our procedure is something of a compromise between extreme views. The scales shown in Tables I and II have been found in continuous practice to be well matched to the capabilities of our panel members, and to provide an acceptable balance between sensitivity and consistency. In the absence of practical methods of testing the efficacy of an arbitrary scale of measurement, these scales appear to be adequate for our needs. It is considered advisable, however, to adjust the marks to compensate for the absence of zeros in the tenderness and juiciness scales when testing for significant differences between treatments. Mean scores however are always given in terms of the actual scale units, without adjustment, and mean scores of zero are possible and meaningful.

This system of scoring was being developed at much the same time as that for wet white fish[6] and the differing natures of the two systems is a reflection of the differences between the problems being investigated. In the case of fish, the object of study was to examine deterioration of quality with time, and Shewan *et al.*[6] were able to specify an order of occurrence of distinguishable flavours and textures, and to relate this to the age on ice of the fish after catching. There was therefore an external continuum measured in fundamental, equal units, against which the arbitrary scoring system could be calibrated and adjusted. No such parallel was possible for beef, since the purpose in general was to examine the effects of production variables and not to study deterioration, and so the only way of judging the efficacy of the scoring system was by empirical experience. It may be that some different form of scale would give better results, but detailed comparisons of the effectiveness of a number of different scales were impracticable with the resources and time available for preliminary methodological studies.

Statistical considerations

In the investigations, some attempt has been made to eliminate one particular source of variation by statistical analysis rather than by experimental design. This is the extent to which individual tasters will vary in their overall level of scoring in any particular investigation. Importance is attached to the difference between the scores given by a taster to different samples rather than to their absolute values. A taster may always have a ' bias ' towards the use of higher or lower scores compared with other tasters, and experimental errors are reduced if due allowance can be made for these biases. Such a reduction of experimental errors is valid when the tests are of the ' analytical ' type. Four methods have been used to achieve this purpose :

* This phrase has been used by American workers, and we cannot think of a better.

Table I

Roast beef score card

DO NOT USE HALF MARKS Taster :

Sample no.	A.	B.	C.	D.
1. Lean meat flavour				
2. Juiciness				
3. Tenderness				
4. Degree of doneness [Judged by colour and texture]				
5. Fat off-flavour				
6. Lean off-flavour				

Notes

1. *Meat flavour.* Score intensity of meat flavour o to 5, o being tasteless or insipid, and 5 strong flavour.

2. *Juiciness*

Very juicy	+5
Juicy	+4, +3, +2
Sl. juicy	+1
Sl. dry	−1
Dry	−2, −3, −4
Very dry	−5

3. *Tenderness*

Very tender	+5
Tender	+4, +3, +2
Sl. tender	+1
Sl. tough	−1
Tough	−2, −3, −4
Very tough	−5

4. *'Degree of doneness'*

Grossly overdone	+5
Overdone	+4, +3, +2
Sl. overdone	+1
Neither underdone nor overdone	o
Sl. underdone	−1, −2
Underdone	−3, −4
Grossly underdone	−5

5 and 6. *Off-flavours* (specify with adjective)

No off-flavour	o
Degree of off-flavour	−1, −2, −3, −4
Very strong off-flavour	−5

[Sl = slightly]

(*a*) the extraction of a mean square 'between tasters' in an analysis of variance—comparable to the calculation of a source of variation 'between blocks' in a randomised block experiment. Strictly this method is only applicable where all tasters have been present at all tasting sessions;

(*b*) where 'occasional absenteeism' has been infrequent, missing plot techniques have been used to fill in the missing readings, and method (*a*) employed;

(*c*) where one or two tasters have been absent from several sessions, their scores have been excluded entirely, unless this has meant reducing the effective size of the panel below five members;

(*d*) where occasional absenteeism has been frequent, it has sometimes been possible to pair the individual scores. For example, in an investigation of the effect of hexoestrol implantation on eating quality, the four samples presented at any one tasting session consisted of two treated and two untreated samples. The scores given to the treated samples by each individual taster on each occasion were subtracted from the scores given by the same taster to the untreated samples on the same occasion, and these differences assessed for significant departure from zero. This is a well-known use of the 't'-test, valid only when a unique pairing is possible. It automatically disregards differences between pairs, as opposed to differences within pairs, that is, taster bias in this application.

(*e*) where none of these methods is possible, 'biases' are included in the experimental errors which are thus inflated in consequence. This has the effect of decreasing the sensitivity of tests of significance.

Table II

Stewed beef score card

Sample no.	TASTER: A	B	C	D
Meat flavour				
Meat toughness / tenderness				
Liquor flavour				
Liquor ' body '				
Comments on liquor, e.g., fatty, greasy, meaty, dark, etc.				

Meat toughness (tenderness)

+5 Very tender
+4, +3, +2 } Tender
+1 Sl. tender
−1 Sl. tough
−2, −3, −4 } Tough
−5 Very tough

Meat flavour and liquor flavour

Note: Any score of −1 or less must be accompanied by an adjective. You may give two scores for meat flavour and two scores for liquor flavour if you wish.

+5 Very strong meat flavour
+4, +3, +2, +1 } Degree of meat flavour
0 Tasteless
−1, −2, −3, −4 } Intensities of foreign flavour
−5 Very strong foreign flavour

Liquor ' body '

+5 Thick (meaty)
+4
+3
+2
+1
0 (water)

Method of presentation

Meat is naturally heterogeneous, and it is realised that there is a possibly large sampling variation even within any given joint. Not only does this necessitate the examination of a large number of samples in order to achieve any degree of statistical significance, but it may also lead to serious disagreement between tasters in the scoring of any one joint. In several tests, the meat was minced before being presented, but it was generally agreed by the tasters that mincing introduced a slight ' metallic ' flavour and it was thought that mincing would interfere with the recording of any differences in tenderness that may otherwise have been apparent. The practice of mincing was therefore discontinued. Slices of roast beef cut off a joint, on the other hand, would maximise the interference of sampling error with taster agreement, and a compromise procedure was adopted in which definite muscles such as the *longissimus dorsi*, *psoas major* or *semitendinosus* were dissected out and cut into cubes of about 1 cm. side after removal of the outer layer. After randomisation, the cubes were placed in glass dishes, each taster being presented with a separate sample consisting of five or six cubes of lean and a representative sample of cubes of the subcutaneous fat.

The preliminary training period included several tests designed to discover optimum methods of cooking and of presenting the samples to the panel. It was considered that the method of presentation which resulted in most improved discrimination or consistency, or both, was to be preferred. One point to which considerable attention was given was the comparison of hot tasting with cold. One half of each of several samples was presented to the panel immediately

after the meat had been cooked, the other half being allowed to cool to room temperature before being presented. In all other respects, the procedure was the same. Comparison of the scores given by the tasters to hot and cold samples showed that cold tasting was preferable. Table III gives an analysis of variance for three treatments and seven tasters, for each characteristic except meat flavour (which showed no significant differences between treatments). A higher variance ratio was obtained with cold tasting, due partly to a higher degree of discrimination and partly to a lower error mean-square. In this particular test there were no replicate samples, so that the error mean-square, being an interaction, reflects the extent to which tasters disagreed with each other in their comparative scoring of the three samples (treatments). The mean-square for tasters was generally greater for cold tasting, and this was thought to be due to the fact that the panel had already undergone some considerable training with hot samples, and a measure of agreement between their scores had been achieved. After similar training and discussion, using cold samples, these biases were considerably reduced. Cold tasting has great advantages over hot tasting from the practical point of view because it allows greater freedom in the timing of tasting sessions. With hot tasting, if the temperature of cooking is standardised, the first samples to come out of the ovens have to be kept hot until the others are ready and the panel has to be assembled at exactly the right time. The usual procedure is to cook the meat during the afternoon of the day before a panel session, and keep it overnight in a household refrigerator. This applies only to the roast samples.

It is realised that cold roast beef may arouse a different set of sensations in the mouth from hot roast beef. Nevertheless, for the purpose of the comparisons intended to be studied when these investigations were begun, it was thought that results with cold beef would be sufficiently meaningful in themselves. Some deteriorative off-flavours, particularly rancidity in the fat, may be more easily detectable in hot meat, but no such effects were expected. In some recent investigations, flavour effects were more likely, and samples were tasted whilst still hot. Stewed beef is always tasted hot, and in those cases where it has been advisable to taste hot roast beef, some degree of control over the cooking process has been sacrificed in order to ensure that the joints are ready at the same time. Hot tasting is, however, the exception, and cold tasting the rule.

It was later found with other foodstuffs that the time when the tests took place could also influence the results.[7] Using the same criteria (greater discrimination and lower experimental errors) it was found that results obtained in the morning were better than those obtained in the afternoon. It is therefore possible that the better results for cold beef apparent in Table III were due to the fact that cold tasting was usually done in the morning and hot tasting usually in the afternoon. This was almost inevitable with the cooking temperature (and consequently cooking time) adopted during these tests. Hot tasting, if carried out in the morning, might lead to still more sensitive results, but in view of the practical convenience of cold tasting, and the certainty that cold tasting in the morning gave better results than hot tasting in the afternoon, the point has not been investigated further with beef.

Standardisation of cooking

In studies of the influence of production variables on palatability, the cooking process must be rigidly standardised, and it is not easy to exercise scientific control over the art of cooking. The experimenter is faced with a choice of cooking the meat for a standard time (or a standard time per unit weight) or of cooking the meat until it reaches a fixed temperature. He cannot do both. In these experiments the latter alternative was adopted because it was thought to be more amenable to standardisation of the method and likely to interfere least with inherent differences in the meat. Experiments were devoted to a comparison of different oven temperatures and different final internal temperatures of the meat. The purpose of these tests was to find that combination of temperatures which allowed the maximum discrimination by the panel, and achieved a degree of cooking acceptable to the members and of reasonable uniformity through the whole joint. It was found to be impracticable to adapt a single oven to cook more than one joint at a time, with any satisfactory degree of uniformity. No skewers are used in dressing the joints for cooking because they produce heat-transfer irregularities.

Table III

Comparison of hot and cold tasting

Characteristic scored	Source of variance	Degrees of freedom	Tasted hot		Tasted cold	
			Mean square	Variance ratio	Mean square	Variance ratio
Fat off-flavour	Between treatments	2	0·95	2·6	1·19	4·6
	Between tasters	6	1·05	2·9	1·52	5·8
	Error (residual)	12	0·36	—	0·26	—
Meat flavour	Between treatments	2	0·05	—	0·25	—
	Between tasters	6	0·77	2·2	2·13	5·9
	Error (residual)	12	0·35	—	0·36	—
Juiciness	Between treatments	2	0·58	2·6	1·75	12·5
	Between tasters	6	1·08	4·9	2·31	16·5
	Error (residual)	12	0·22	—	0·14	—
Tenderness	Between treatments	2	1·00	4·0	1·96	12·3
	Between tasters	6	2·70	10·8	1·31	8·2
	Error (residual)	12	0·25	—	0·16	—

Measurement of temperature

All cooking was carried out in gas-heated ovens and the following method was used to standardise cooking conditions. A copper/constantan thermocouple, with the hot junction embedded in the centre of a 1 in. dia. solid, blackened, brass sphere, and mounted in a fixed position in the oven (i.e., centrally and 4 in. from the top of the oven) was used for temperature measurement. The temperature at the centre of the brass sphere was maintained by adjustment of the oven thermostatic regulator, and this temperature is referred to here as the oven temperature.

The internal temperature of the joint was taken to be the temperature indicated by a nichrome/constantan thermocouple, mounted in a stainless steel sheath, with the hot junction as near as possible to the geometric centre of the muscle to be tasted.

All thermocouple wires were taken to a common reference point (vacuum flask containing a mixture of water and ice) from which copper leads were connected to a 12-point rotary selector switch. Thermal e.m.f. were measured with a potentiometer, readings being taken every 15 min., or more frequently as the critical point was reached.

Oven temperature

It was found that, in general, the lower the cooking temperature, the more evenly cooked was the meat, but there was a danger that if heated at too low a temperature for too long, all samples might be cooked to characterless uniformity. Table IV gives some of the earlier results. The flavour tended to be less in joints cooked at a lower oven temperature. Although the results for one of the animals quoted in Table IV (Animal A) are not consistent, the results for the other animal support the expectation that, the higher the oven temperature within this range, the less juicy and the less tender was the cooked product. What mattered in these tests was not the absolute level of any of these characteristics, but the effect on discrimination and consistency of scoring, and it was found that the higher the oven temperature, the greater was the likelihood of disagreement between tasters presumably because of relatively uneven cooking. An oven temperature of 300° F was ultimately adopted as standard, since it was found that the loss of flavour (compared with that at higher temperature) was not enough to reduce discrimination to any measurable extent. This oven temperature is lower than most British housewives would use for roasting beef. Workers in America have used oven temperatures as low as 230° F and as high as 500° F. A review of the literature on this point has been published elsewhere.[8] Common consumer practice in this country is to roast beef on a time per unit weight basis (e.g., 20 min. per lb. plus 20 min.) and it is interesting to note that results collected over a number of years, using a standard oven temperature and a standard final internal temperature, show a very considerable range in time of cooking per unit initial weight from 5 min. per 100 g. to 22 min. per 100 g. with an average of about 12 min. per 100 g., all data being for joints within half a pound of 3 lb. raw boned weight initially.

Table IV

Effect of varying oven temperature

	Oven temperature, °F	Animal A	Animal B
Meat flavour	300	2·8	2·8
	350	3·0	3·0
	400	3·3	3·0
Tenderness	300	+2·8	+1·4
	350	+1·2	−0·3
	400	+3·3	−0·7
Juiciness	300	−1·7	+1·4
	350	−0·7	+0·9
	400	−1·9	+0·7

Final internal temperature

It was found in early tests that higher final internal temperature at constant oven temperature gave a product with less flavour, less juiciness, and less tenderness. Some of the results obtained are given in Table V. There was, however, a practical lower limit to the final internal temperature, because some tasters refused to eat meat which appeared to be grossly undercooked, and it was established that a final internal temperature of 160° F gave a product that was sufficiently well cooked to be acceptable to all tasters, whilst still retaining sufficient juices to permit good discrimination.

Summary of present technique

(a) *Roasting* (for tasting cold)

Rib joints are boned, rolled and weighed before being cooked in open roasting tins in gas-heated ovens. The ovens are pre-heated to 300° F before the meat is inserted and a separate oven is used for each joint. Thermocouples are inserted into the centre of each joint and the temperature read every 15 min. until the joint reaches a temperature of 160° F when it is removed. In practice it is found that the internal temperature of the joint rises a further 3–5° F after removal from the oven. The cooked joints are weighed and placed in a domestic refrigerator overnight. Next morning they are again weighed, the *longissimus dorsi* muscle is dissected out and cut into cubes of approximately 1 cm. side and these cubes are divided at random among the judges, together with a random sample of the subcutaneous fat.

(b) *Stewing*

With stewing joints, the procedure is to trim the meat of gross amounts of fat and cut the remainder into cubes of approximately 1 in. side. Samples of 680 g. are weighed into casseroles and 400 ml. of cold tap water added to each. The casseroles are covered, and the meat stewed for $3\frac{1}{2}$ h. in gas-heated ovens at 300° F. Each sample is stirred after 1 h. cooking. Each member of the panel is given a portion of meat and liquor, served hot, for separate assessment.

(c) *Presentation*

All judging is done in separate cubicles, in a room specially designed for the purpose, to ensure independence of judgment by the tasters. The judges, who are chosen for their acuity and consistency, have no foreknowledge of the purpose of the tests. All samples are coded at random. Not more than four samples are judged at any one session and no more than one session is held on one day, except in exceptional circumstances. All panel sessions are held in the morning. Bread and water are provided as palate cleansers, and the tasters are encouraged to take their time and to re-taste frequently.

Interpretation of results

The taste panel described is an analytical panel. It affords a means of assessing the eating qualities of cooked meat. The panel does not measure acceptability to consumers, or provide any single overall estimate of palatability. Consumers vary in the importance they attach to the several qualities and in their conception and perception of optimum intensities for each. Much existing knowledge of what makes for optimum palatability of beef is based on subjective

Table V

Effect of varying final internal temperature

	Final internal temperature, °F		
	170°	159°	143°
Meat flavour	3·5	3·9	4·1
Juiciness	3·5	4·3	4·5
Tenderness	4·0	4·4	4·8

Note. The scales used here were 0–8, a higher mark indicating more flavour, more juiciness, and more tenderness

experience of a non-scientific nature. As far as we know, no systematic work on beef palatability has been published in the United Kingdom, although Callow[9, 10] gives a very brief account of his work on the subject.

It may be argued that if the whole of palatability is divisible into three components—texture (toughness or tenderness), flavour and juiciness—then palatability could be represented by a combination of the separate estimates for the three components. This would be possible if the score scales for each attribute could be shown to have some measurable relation to each other. There is, however, no satisfactory frame of reference.

The results of analytical taste panels can only be related to consumer acceptance by making direct comparisons between such results and those of consumer acceptance studies. Otherwise the use of taste panel results to predict consumer acceptance must rest on presumptions as to the weight attaching to particular aspects of quality in determining acceptability to the consumer. The quality characteristics assessed in these tests are, it is believed, those that matter to consumers, but it is preferred not to derive any single index of palatability from the data available.

We are satisfied that the methods which have been described are adequate to provide meaningful results. They have been used in a series of investigations which will be the subject of other papers. They have led to significant conclusions and, although they may not have revealed with certainty all that might have been found, it is fair to conclude that those facts which have been established are at least of greater significance than those which have escaped detection.

The methods of cooking and presentation that have been used in these investigations may not be the best possible. But as the preparatory work described in this paper took one year to complete, it was felt that sufficient experience had been gained for these methods to be used in practice.

Internal tests of sensitivity have indicated that the methods used have been moderately successful. Without preliminary trials in methodology, the sensitivity and accuracy of our results would have been less.

Acknowledgments

This paper refers mainly to an early stage of work which has been a team operation continuing over many years. There have been many team changes. The authors wish to acknowledge the leading parts taken by former members of this Division, notably Mr. N. E. Holmes and Mr. H. R. Hinton. They also make grateful acknowledgment of the most important contributions made by the taste panel members, without whose conscientious co-operation no progress would have been possible.

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THE COLLECTION AND IDENTIFICATION OF THIOLS AND DISULPHIDES

By A. R. FOLKARD and A. E. JOYCE*

Following the evolution of sulphur compounds from cultures of marine organisms in methionine solution, the collection of thiols and disulphides in mercuric cyanide and mercuric chloride was studied. The subsequent conversion of the mercaptides and mercury complexes to 2,4-dinitrophenyl sulphides was found to be possible by decomposing the mercury compounds with acid and collecting the mercaptan in caustic ethanol and then treatment with 1-chloro-2,4-dinitrobenzene. A method for the chromatography of the 2,4-dinitrophenyl sulphides on paraffin-impregnated paper was devised and studied.

Introduction

During attempts to separate and identify the metabolites of marine organisms cultured with methionine, an odour, closely resembling that of organic sulphur compounds, was observed.[1] This was also noticed when samples of marine organisms were treated with a solution of 10% caustic soda. These volatile sulphur compounds were collected successfully in mercuric cyanide and mercuric chloride according to the method of Challenger.[2] The further identification of the alkali-decomposable precipitate[3] from mercuric chloride, by paper chromatography of the regenerated sulphide as the sulphidimine[4] was found to be satisfactory. This method of Challenger & Leaver has recently been extended and improved by the use of *p*-nitrobenzenesulphonchloramide, $NO_2 \cdot C_6H_4 \cdot SO_2N(Cl)Na$, instead of chloramine T.[5]

There are, however, few methods, apart from the melting points of the mercaptides of mercury and lead, for the further identification and separation of thiols. The thiols were either given off as thiols from the cultures and decomposition experiments, or were obtained from the acid decomposition of mercury complexes formed in mercuric chloride and mercuric acetate solution from fission of the disulphides.[2, 3]

The reaction of thiols with mercuric cyanide leads, it is supposed,[2] to the formation of the insoluble complex $(RS)_2Hg$ and $(RS)_2Hg \cdot Hg(CN)_2$. By boiling with water[3] the mixture is converted to the pure mercaptide which may be recrystallised to give a well defined melting point. If the material for examination consists of mixture of thiols, the separation could only be effected by gas chromatography.

In earlier work 1-chloro-2,4-dinitrobenzene was used by Boost and others[6] to prepare 2,4-dinitrophenyl sulphides from thiols; these sulphides were also prepared by Carson & Wong[7] from lead mercaptides by refluxing with the reagent named. The latter authors[8] chromatographed the 2,4-dinitrophenyl sulphides on silicic acid and fluorescent zinc sulphide. It was felt, however, that paper chromatography of the 2,4-dinitrophenyl sulphide might be more suitable. It was not possible to prepare crystalline 2,4-dinitrophenyl sulphides by refluxing 1-chloro-2,4-dinitrobenzene with a mercury mercaptide.

The crystalline 2,4-dinitrophenyl sulphides were finally prepared, both from the mercury mercaptides and the mercury complex resulting from disulphide fission, by decomposition with acid and aspiration of the acid-free thiol vapours into ethanolic sodium hydroxide, and subsequent addition to an ethanolic solution of 1-chloro-2,4-dinitrobenzene.

The same method proved satisfactory for lead mercaptides, and was also found to be suitable for preparing a mixture of 2,4-dinitrophenyl sulphides from a mixture of mercury mercaptides of methane-, ethane-, propane- and butane-thiol. This mixture of mercaptides was prepared by aspirating mixed vapours from authentic samples of thiols into mercuric cyanide. If the quantity of mercaptide available was very small, then the alcoholic solution of the reactants could be spotted on to the paper, the reagent and any 2,4-dinitrophenol separating readily from the 2,4-dinitrophenyl sulphides. Systems for the paper chromatography of nitro-derivatives have been described by Boyland[9] and Asatoor.[10, 11] The solvent system used by Boyland for nitro-derivatives of sulphur-containing amino-acids was not suitable for the present work, but his spray reagents gave good results. Titanous chloride was used for reduction of the nitro-

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groups; the resulting amino-compounds were then condensed with *p*-dimethylaminocinnamaldehyde. This procedure gave a deep purple colour with 2,4-dinitrophenyl sulphides, which changed to orange pink in 2 days, and was stable for several weeks. The reversed-phase system of Asatoor[10] was found, with slight modification, to be suitable for the 2,4-dinitrophenyl sulphides of aliphatic thiols. As stated by Asatoor, difficulty was experienced in obtaining reproducible R_F values on paraffin-impregnated paper, and the rates of movement were therefore related to the movement of methyl 2,4-dinitrophenyl sulphide.

Disulphides do not react with mercuric cyanide but decompose slowly with mercuric chloride, according to Challenger[2, 3] by the reaction given:

$$2R{\cdot}S{\cdot}S{\cdot}R + 6HgCl_2 + 2H_2O \rightarrow 3R{\cdot}S{\cdot}HgCl,HgCl_2 + R{\cdot}SO_2H + 3HCl$$

Disulphides also react with mercuric acetate in a similar way to give the soluble compound $R{\cdot}S{\cdot}Hg{\cdot}O{\cdot}CO{\cdot}CH_3$.[2] They may be precipitated by the addition of sodium chloride[12] to give an insoluble compound which Birch & McAllan believed to be RS·HgCl. These compounds $R{\cdot}S{\cdot}HgCl,HgCl_2$ and R·S·HgCl can be treated in the same ways as the mercaptides obtained with mercuric cyanide and thiols, to give 2,4-dinitrophenyl sulphides. These in turn can be examined by paper chromatography on paraffin-impregnated paper.

Experimental

Preparation of mercury mercaptides from thiols

Small quantities (0·1 ml.) of individual thiols or a mixture of them (obtained as authentic samples from B.D.H., Messrs. Light and Eastman Kodak) were placed in a simple bubbler tube and aspirated with nitrogen which had previously been passed through mercuric chloride solution (3% w/v). The stream of nitrogen from the tube containing the thiol was passed into two tubes of mercuric cyanide solution (4% w/v). The white precipitate which formed in the latter was filtered off, boiled with a small quantity of distilled water, cooled, filtered and recrystallised from ethanol or ethyl acetate. Melting points are shown in Table I. Propane-2-thiol reacts slowly with mercuric cyanide and the mercaptide is liquid at room temperature before recrystallisation. It tends to interfere with the collection of the other thiols; instead of a white floccular precipitate a somewhat waxy material is formed.

Preparation of mercury complexes from disulphides

Small quantities of individual disulphides or of disulphide mixtures (obtained from the same sources as the thiols) were aspirated in the same way, first into mercuric cyanide to remove any trace of thiol, then into mercuric chloride (3% w/v). At least eight collecting tubes were necessary owing to the slow nature of the reaction.[2] The precipitates were very insoluble in common organic solvents and the methyl compound had a high and indefinite melting point.

Disulphides were also aspirated into mercuric acetate (4% w/v) and in this case only three tubes appeared to be necessary for complete absorption. The insoluble complex was precipitated from the mercuric acetate solution by the addition of a few drops of saturated aqueous sodium chloride. Blank tests were conducted with mercuric acetate and sodium chloride solutions to test for mercurous salts.

Preparation of mercury co-ordination compounds of sulphides

Authentic samples of sulphides were aspirated in the same way, first into mercuric cyanide to remove any trace of thiol, then into mercuric chloride (3% w/v). The precipitate was decomposed by alkali according to the method of Leaver & Challenger[4] for preparation of the sulphidimines. These were subsequently identified by paper chromatography.[4]

Preparation of crystalline 2,4-dinitrophenyl sulphides from mercury mercaptides and mercury complexes (from disulphides)

Mercury mercaptide, or mercury complex from an individual thiol or a thiol mixture (in the case of mixtures a little more mercaptide was required) or a disulphide, was placed (0·05 g.) in a tube (1 cm. × 4 cm. internal dimensions). This was connected in series with similar tubes to form a gas absorption train. The first absorption tube after the tube containing mercaptide

was half filled with distilled water to remove any hydrogen chloride fumes. The following two tubes each contained 2·5 ml. of ethanol and approximately 0·015 g. of solid sodium hydroxide. To the tube containing the mercury compound was added 1 ml. of 8N-hydrochloric acid and the gas inlet tube was arranged as to be 1 cm. above the level of the hydrochloric acid. The tubes were connected to each other and to a source of purified nitrogen. The tube of acid and mercury compound was then gently warmed till all the compound had dissolved. It was then allowed to cool and the gas flow started. Gentle heat was applied to the connecting pieces and to the distilled water to prevent condensation of any of the thiols of higher boiling point. After some 15 min. the gas flow was stopped. The two tubes of ethanolic sodium hydroxide were now added slowly to separate volumes (1·25 ml.) of alcoholic 1-chloro-2,4-dinitrobenzene (saturated at 25°) and the inside of the gas inlet tube, in each case, was rinsed with a little of the mixture. The solutions were then cooled in ice. The crystals were filtered and to the filtrate was added a further small volume of the reagent. The filtrate was then cooled, and if no crystals separated it was discarded. The 2,4-dinitrophenyl sulphides were recrystallised from ethanol.

It was found that, with the mixtures, the thiols of lower boiling point tended to accumulate in the second tube. If, when a mixture of thiols was being examined, one thiol was present in a much smaller proportion than the rest, the corresponding dinitrophenyl sulphide occasionally failed to crystallise. In such instances the filtrate from the reaction mixture was also spotted on the paper. As little as 5 mg. of mercury compound would give crystals of 2,4-dinitrophenyl sulphide by this technique. If 1 mg. of mercury compound was used with 1 ml. of ethanol and 0·010 g. of alkali in two tubes and 0·25 ml. of reagent reacted with the contents of each tube, spotting of the reaction mixture gave detectable spots of 2,4-dinitrophenyl sulphides for an equal mixture of four thiols. In the case of 1 mg. of mercaptide it was found preferable to use slightly shorter tubes and to dispense with the tube of water for absorption of hydrochloric acid vapour. When the reaction mixture was spotted on the paper, the unreacted 1-chloro-2,4-dinitrobenzene and 2,4-dinitrophenol separated from the 2,4-dinitrophenyl sulphides.

Preparation of crystalline 2,4-dinitrophenyl sulphides from thiol vapours

The 2,4-dinitrophenyl sulphides were prepared direct from mixed or individual thiol vapours by passing nitrogen over very small amounts of liquid thiol placed in the tube normally reserved for the mercaptide. The tube of distilled water was discarded, but the tubes of ethanolic sodium hydroxide were retained. The reaction was carried out in exactly the same way as for the mercury mercaptides, the ethanolic sodium hydroxide being poured slowly into the reagent and then cooled.

Paper chromatography of 2,4-dinitrophenyl sulphides

Strips of Whatman No. 3 MM paper (23 × 57 cm.) were placed in a shallow tray (25 × 60 cm.) containing liquid paraffin (sp. gr. 0·83–0·87) dissolved in light petroleum (b.p. 80–100°). The solution was made to flow over both surfaces of the paper by gently tilting the tray and by turning the paper. The paper was then allowed to dry in air. The solvent system of Asatoor[10] was slightly modified, the amount of methanol being increased to give chloroform/methanol/water/liquid paraffin in the proportion 15 : 20 : 9 : 6. The mixture was set aside for at least 8 h., by which time the two phases had become clear. In all cases the lower phase was poured into a dish and left on the floor of the tank.

The 2,4-dinitrophenyl sulphides were applied to the paper dissolved in chloroform or alcohol if the quantity of mercaptide were small. The chromatograms were run by the descending technique for 8 h. at 20°. They were subsequently dried in warm air and sprayed first with titanous chloride (15% w/v), then with *p*-dimethylaminocinnamaldehyde (2 g. in 100 ml. of 6N-hydrochloric acid and 100 ml. of ethanol). The 2,4-dinitrophenyl sulphides showed up as dark purple spots on a pale grey-purple background, changing during 2–3 days to orange-pink spots on a greenish-blue background. 2,4-Dinitrophenyl isopropyl sulphide gave a distinct orange-pink colour on first spraying. On examination of the freshly sprayed or 3-day-old chromatograms by ultra-violet light, the spots showed up as bright pink. The spots from 1-chloro-2,4-dinitrobenzene and 2,4-dinitrophenol differed in the shade of pink compared with

that from the 2,4-dinitrophenyl sulphide. Ultra-violet light without spraying also showed up the 2,4-dinitrophenyl sulphides but not as clearly.

Collection of thiols and disulphides from biological materials

One of the following—air, oxygen or nitrogen—was used as the gas for aspirating through cultures or reaction mixtures for the removal of volatile sulphur compounds, the choice depending on the need for aerobic, anaerobic or inert gaseous conditions. Where thiols were given off from cultures and air was used for aspiration, the tendency for thiols to be oxidised to disulphides[2] was not noticed, as was evident from the absence of a precipitate in mercuric chloride.

The gas was first passed through conc. sulphuric acid, mercuric chloride (3% w/v) and boiled distilled water, to remove traces of sulphur compounds and micro-organisms.[13] If, in a decomposition experiment with sodium hydroxide, ammonia was given off in quantity, a tube of 0·5N-hydrochloric acid was placed immediately after the reaction tube, before the mercuric cyanide, to remove the ammonia and prevent the formation of aminomercuric chloride NH_2HgCl.[14] In the absence of ammonia the hydrochloric acid tube was omitted and the gas stream aspirated through mercuric cyanide solution (4% w/v), then into mercuric chloride solution (3% w/v). When carbonyl compounds were to be investigated, two or more tubes of 2,4-dinitrophenylhydrazine were placed between the last mercuric cyanide tube and the first mercuric chloride tube. The number of tubes of any reagent used was arranged so that one tube was free from any reaction product before the gas passed to the other tubes in the series. Where disulphide formation was indicated by (*a*) the inability of mercuric chloride to remove all the odour from the gas stream or/and (*b*) the slow formation of precipitation in a second and third mercuric chloride absorption tube, three tubes containing mercuric acetate solution (4% w/v) were placed after the mercuric chloride tubes.

The precipitates formed were examined by the procedure outlined above. Those in the mercuric cyanide solution were examined for thiols and those in the mercuric chloride tube for sulphides[4] by decomposition with alkali. The precipitate obtained from the mercuric acetate by the addition of sodium chloride was decomposed with acid for detection of thiols derived from the fission of disulphides. Precipitates in the mercuric chloride, derived from disulphide, were examined by acidifying after decomposition with alkali. In general the amount was small compared with that obtained from the mercuric acetate.

Results

The melting points of the mercaptides, the 2,4-dinitrophenyl sulphides and the R_{methyl} values for the 2,4-dinitrophenyl sulphides are set out in Table I. The R_{methyl} values are based on the mean of at least 24 determinations from both individual and mixed dinitrophenyl sulphide preparations. The separation between butane-1-thiol and 2-methyl-butane-1-thiol was not as good as was desired; the separation between the straight chain compounds was satisfactory. The limit of detection would appear to be in the order of 0·25 μg. per sq. cm.

Discussion

The paper chromatography of the 2,4-dinitrophenyl sulphides of the C_1 to C_4 thiols on paraffin-impregnated paper and the reduction of the nitro group followed by condensing the resulting amino-group with *p*-dimethylaminocinnamaldehyde, has been found to be a useful technique for separating and identifying this group of compounds. The aspiration of disulphides into 4% mercuric acetate solution proved a suitable way of collecting these compounds; this could be accomplished with fewer absorption tubes than when mercuric chloride was used. If a thiophen compound were encountered in a biological system, then this would need to be taken into consideration when investigating any precipitates formed in the mercuric acetate. Some thiophen compounds, e.g., 2-methylthiophen, form insoluble compounds with mercuric acetate[13] and these if present could be removed before the addition of sodium chloride solution. Compounds of the tetrahydrothiophen type[12, 15] form soluble addition compounds with mercuric acetate and would be precipitated, along with the complexes derived from disulphide, on the addition of sodium chloride. Since the compounds of tetrahydrothiophen, formed in the mercuric acetate solution and precipitated with sodium chloride, are unstable to alkali they could be

Table I

Melting points of mercury mercaptides, 2,4-dinitrophenyl sulphides and R_{methyl} *values*

	m.p. of mercury mercaptide from 4% w/v mercuric cyanide solution			m.p. of 2,4-dinitrophenyl sulphide			R_{methyl}
	Found	Literature	Reference	Found	Literature	Reference	
Thiol							
Methyl	177	175	18	128	128 127	17, 6, 16	1·00
Ethyl	75·5	76	2, 18	114	115 114–115	17, 6, 16	0·77
1-Propyl	70	67	13	85·5	81 85–86·6	17, 6, 16	0·58
2-Propyl	75·5			93	93·5–95 95 94·5	16, 17, 6	0·66
1-Butyl	85	83–84	18	65	65·5–66 66	16, 6, 17	0·43
2-Methyl-1-propyl	91·5			72·5	76 74·5–75	6, 16, 17	0·47
1,1-Dimethylethyl		Unstable		109·5	109–111	17	0·53
n-Pentyl	64			79	80	6, 16	0·30
n-Hexyl	55			75	73·5–75	6, 16	0·22
Disulphide							
Dimethyl				128	128 127	17, 16, 16	1·0
Diethyl				114	115 114–115	17, 16, 16	0·77
1-Chloro-2,4-dinitrobenzene				52	α53, β43, γ27	19	1·11
2,4-Dinitrophenol				112	111·6–114	19	1·24

* $R_{methyl} = \frac{\text{distance travelled by 2,4-dinitrophenyl sulphide}}{\text{distance travelled by methyl 2,4-dinitrophenyl sulphide}}$

removed from the precipitate by alkali decomposition ; acidification would then release the thiols derived from disulphides in the normal way.

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VARIATIONS IN THE PROPORTIONS AND IODINE VALUES OF FATS AT DIFFERENT LOCATIONS IN THE ENDOSPERM OR EMBRYO

By A. R. S. KARTHA

Variations in the proportions and iodine values of fats at different locations in the endosperm or embryo were studied for several different types of seed.

The variations in oil content and iodine value occur in systematic patterns with reference to the centre of the seed or in some cases the centre of each cotyledon. The patterns differ with different varieties of seed, in some from the centre to the periphery, in others along the vertical axis. There appears to be no connexion between the patterns of iodine value and variation in oil content.

Introduction

Reserve fats are elaborated and stored in different parts of plants, but more commonly in tissues connected with the fruit, namely pericarp, testa and endosperm or embryo. With the exception of olive oil, palm oil, stillingia tallow and a few other fats, all commercial vegetable fats are endosperm or embryo fats. It has long been known[1] that the different fruit tissues can show wide differences in the proportions of fats they contain and also large quantitative and sometimes even qualitative differences in the compositions of the component acids of the fats they contain. No record has yet appeared in the literature concerning variations in the proportions and properties of fats at different locations in any single fat tissue from the fruit, for example from different locations of the fruit coat, endosperm or embryo : this might possibly be due to the fact that the individual tissues have so far been assumed to be uniform in fat contents as well as component acid composition of fats.

The development of a simple cold percolation method[2, 3] for quantitative isolation of fats from very small amounts of tissues made it comparatively easy to test the uniformity of fat content or otherwise of individual fat tissues. The first exploratory study was done with coconut kernel which was separated into top, middle and bottom one-third layers and the oil contents estimated.[4] The oil contents increased from 37 to 41% for the top one-third to 64–68% for the bottom one-third, the middle layers showing intermediate values. The results of a more systematic investigation of variation of fat contents and iodine values of fats from different locations of the endosperms or embryos of a number of seeds are reported in this communication.

Experimental

The variations were studied on a vertical axis of the endosperm or along the middle of the inner faces of the cotyledons and on a horizontal axis radially at the middle of the endosperm or at the middle centre of the cotyledons. The approximate position of the zones examined are shown in Fig. 1 where the vertical zones are numbered I, II and III and the horizontal zones II, IV and V. In cases where the cotyledons were very thin, examination on the horizontal axis (radially) was limited to the inner and outer zones (II and V) alone. The sectionings were throughout done by hand on freshly cut air-dry seeds with safety-razor blades. The slices from the selected zones were subsequently dried to constant weight in an air-oven at 60° and stored in desiccators over fused calcium chloride before analysis which was conducted with the least possible delay in every case. The relative proportions of the sections were not determined

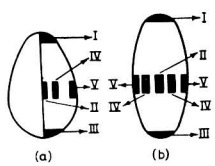

FIG. 1.—*Section of seed showing approximate position of zones examined*
(a) non-endospermic (b) endospermic

as a rule, as the studies were more qualitative than quantitative. The fat contents were determined by the cold percolation method after the seed sample had been ground with excess (6–10 times its weight) of anhydrous sodium sulphate and glass powder: sulphuric ether freshly distilled over alkali was used as solvent throughout.[2, 3] Iodine values were determined by Hanus' technique, reaction time 30 min. and reagent uptake not more than 33%. The figures given are the mean values from three or more determinations in each case.

Results

Downward variations along the vertical axis

Examples of variations in fat contents and iodine values of fats downward along the vertical axis are given in Table I (A and B). The results show that a uniform oil content and iodine value of oil along the vertical axis is a rare occurrence. They also bring out one feature common to all dicotyledonous seeds so far studied, namely that the oil content is invariably lowest and iodine value highest at the topmost portion of the seed, i.e., near the place where the stalk connects the seed to the plant. Apart from this, the other patterns of variations are different for different seeds and for oil contents and iodine values of oil.

Variations in oil content show two separate patterns: (1) in some seeds there is a progressive increase from the top to the bottom (examples 1–3 in Table I A), but this is not the most common pattern; (2) the most common pattern shows a maximum oil content at the middle which decreases both in the upward and downward directions, the value at the bottom end being somewhat higher than that at the top end in most cases (examples 4–9 in Table I A). There is no evidence for the existence of a pattern in which the oil content is minimum at the middle and then increases towards the top and bottom or in which it decreases progressively from the top to bottom.

The variations in iodine value do not appear to have any relation to the variations in oil contents along the vertical axis. Variation in iodine value patterns appear to be of two types: (1) progressive decrease in iodine value from top to bottom. This pattern is seen in seeds showing maximum oil content in the middle (examples 4–6, Table I A) and also in seeds showing progressive increase in oil content from top to bottom (examples 2, 3 in Table I A); (2) the iodine value is minimum at the middle and increases in both directions upwards and downwards. This pattern also is shown by seeds having both types of distribution patterns of oil (example 1 in Table I A for the first type and examples 8 and 9 in Table I A for the second type). No examples have been found of seeds with maximum iodine value in the middle or those showing progressive increase in iodine value from top to bottom.

Maximum variations along the vertical axis found were ~14% for oil content and ~18 units-% for iodine value.

Outward radial variations along horizontal axis in the middle

Results obtained for these variations are given in Table II A and B. With examples 1–7 only the innermost and outermost zones (II and V) were studied. The oil contents increase from the inner to the outer side in examples 1–3 in Table II A and decrease from the inner to the outer side in examples 4–7. In all these cases (1–7, Table II A), the iodine value increases from the inner to the outer side.

Analysis of inner, middle and outer zones (II, IV and V) could be carried out in a few favourable instances (examples 8–13 in Table II A). These show the following variation patterns from inner through middle to outer side:

(i) progressive decrease in oil content from innermost to outermost layers confirming the pattern of decreasing oil content from inner to the outer side already discussed (examples 9–11, Table II A);

(ii) maximum oil content at the middle with decrease towards the inner and outer sides. Of this, however, only one example has been found so far, namely sapota seed (example 12, Table II A).

Patterns of the variation in iodine value observed are of the following types:

(i) progressive increase from the innermost to outermost side (examples 1–8 in Table II B);

Table I

Downward variations, along the vertical axis, of the oil contents and iodine values of oil in some seeds

	Top (I)	Middle (II)	Bottom (III)
A. *Oil content variations*			
1. Hazelnut	65·9	67·0	70·5
2. Apricot, small	48·9	52·7	48·2
3. Apricot, large, sample 1	49·2	50·3	52·8
4. Apricot, large, sample 2	50·5	50·6	52·3
5. Almond	50·5	62·8	60·0
6. Cashewnut	38·2	52·2	47·6
7. Sapota	34·8	30·7	34·6
8. Pistachio nut	56·0	65·0	58·1
9. Groundnut	50·4	56·3	53·7
B. *Iodine value variations*			
1. Almond	98·1	94·7	93·3
2. Apricot, small	104·6	100·9	96·8
3. Apricot, large, sample 1	100·1	99·2	93·8
4. Apricot, large, sample 2	102·0	100·7	95·5
5. Cashewnut	88·9	80·8	78·3
6. Sapota	74·2	71·1	71·2
7. Hazelnut	87·5	81·5	84·4
8. Pistachio nut	110·5	92·2	99·8
9. Groundnut	92·2	85·5	90·8

Table II

Outward radial variations in oil contents and iodine values of oil along the horizontal axis in the middle of some seeds

	Inner (II)	Middle (IV)	Outer (V)
A. *Oil content variations*			
1. Coconut	47·5	—	74·4
2. Arecanut	28·6	—	33·3
3. Hazelnut	67·0	—	68·7
4. Pistachio nut	65·0	—	58·0
5. Groundnut, sample 1	50·3	—	53·0
6. ,, sample 2	50·8	—	45·5
7. Cashewnut	52·2	—	43·7
8. Brazil nut	72·3	68·8	66·4
9. Apricot, small	52·7	53·6	51·5
10. Almond, sample 1	61·2	58·5	52·8
11. ,, sample 2	62·5	61·9	57·8
12. Sapota	30·7	58·2	28·5
B. *Iodine value variations*			
1. Coconut	3·3	—	12·6
2. Arecanut	28·6	—	33·3
3. Hazelnut	81·5	—	87·1
4. Pistachio nut	92·2	—	103·5
5. Groundnut, sample 1	85·5	—	90·8
6. ,, sample 2	92·8	—	99·2
7. Cashewnut	80·8	—	84·6
8. Brazil nut	83·1	91·5	97·2
9. Apricot, small	100·9	100·9	99·0
10. Almond, sample 1	95·6	97·5	93·5
11. ,, sample 2	94·4	96·9	92·9
12. Sapota	71·1	64·2	68·8

(ii) iodine value maximum at the middle, decreasing both towards inner and outer sides (examples 10–11 in Table II B);

(iii) iodine value minimum at the middle, increasing both towards inner and outer sides (example 12, Table II B).

In the studies along the middle horizontal axis also, the patterns of the variation in iodine value do not show any relation to the variations in oil content. Maximum variations observed in this category are ~30% for oil content and ~14 units for iodine value.

More detailed study of variations in the coconut kernel

The patterns discussed above show only the more predominant features involved in variations in oil content and iodine value. That subsidiary changes in the general patterns can exist is shown by the example of a coconut kernel where, after preliminary studies, a detailed minute sectioning of specific areas was undertaken on the basis of the preliminary results. The serial numbers of the zones starting from the top layer of the kernel, the proportions that the zones constitute of the total kernel, as well as the oil contents and iodine values of oil of each zone are given in Table III. These results show that, although there is a general progressive increase in the oil content and iodine value of oil from the top towards the bottom layers, relatively thin layers do exist at the bottom which contain little oil and similarly also a thin layer at the top which shows higher iodine values than the layers coming further down.

Discussion

The composite oil from the total kernel of the coconut reported in Table III had iodine value 9 and the results in Table III show that this oil consists of a mixture of different oils with iodine values varying from 6 to 46.

Results in Tables I–III show that individual fruit tissues are by no means uniform in oil content or iodine value of oil. The general conception that oil from a specified tissue of any particular seed will represent a ' pure ' oil in the sense that its glyceride structure can be

Table III

Distribution of specific layers in the coconut kernel and the oil contents and iodine values of oil of these layers

Layer number	% wt. of layer in total kernel	Oil content %	Iodine value of oil
1 Topmost	2·1	20·7	7·9
2	79·0	63·4	5·9
3	9·3	72·3	11·7
4	6·5	66·0	24·5
5	2·5	43·8	42·6
6 Bottommost	0·5	10·0	46·0

calculated from a single component acid composition according to any of the theoretical conceptions of glyceride structure is hence seen to be untenable. From this point of view perhaps only the oil from a single cell can be considered ' pure ' and all other oil specimens will be mixtures or composites.

These results have some bearing on the study of the glyceride structure of natural fats. Theoretical calculations of the glyceride structure of individual natural fats are made on the assumption that they are ' pure '. In reality they are composite oils and the experimental figures will only be the mean values for a number of fats with proportions of fatty acids varying on either side of the experimental figures obtained for the mixed fatty acids. The difference between the experimental and calculated figures will depend on the average deviation from the mean values. The changes present in the experimental figures will be an increase over the calculated figures in the proportions of trisaturated and triunsaturated glycerides in fats where trisaturated glyceride formation is not restricted, and increase in the proportions of disaturated and triunsaturated glycerides in fats where such restriction occurs. The magnitude will be about 1 unit-% for a saturated acid deviation of ± 5 units-% from the mean values, and higher per unit as the deviation increases. The proportions of glycerides of different composition and configuration will also show differences from calculated values for the same reason. In comparing experimental values with theoretical, consideration has therefore to be given not only to possible experimental errors but also to variations caused by the fat samples being composite.

A simultaneous scrutiny of the variations in oil content and iodine value along the vertical and middle horizontal axes of the same seed shows that in most cases the variations show some definite pattern with reference to the centre of the seed. Thus in the monocotyledons, coconut and arecanut, the oil content and iodine value of oil appear to increase progressively from the central point to the periphery on all sides. Some dicotyledonous seeds, e.g., groundnut and pistachio nut, show a different arrangement, oil content being maximum at the centre and decreasing towards the periphery on all sides, while iodine value is minimum at centre and increases towards periphery on all sides. The sapota seed is unique in that it shows this arrangement with respect to each cotyledon rather than the whole seed. In hazelnut, the iodine value of the oil increases from the centre towards the periphery on all sides as with coconut and arecanut, while the oil content increases towards the outer periphery only at the bottom and middle and decreases slightly towards the top. In apricot (small variety), cashewnut and almond the oil content decreases from the centre towards the periphery on all sides while the iodine value increases from the centre outwards, only towards the top and middle peripheries and shows a decrease towards the bottom : in apricot and almond the variations in iodine value follow the systematic pattern more closely if considered with respect to the centre of each cotyledon rather than the whole seed as in the case of the sapota. In general, it may be said that the oil content and iodine value of oil either decreases or increases progressively from the centre to the periphery either with regard to the whole seed or with regard to the separate cotyledons in some non-endospermic seeds. Alterations in this pattern when observed are found to be limited to small portions of the seeds, namely from the centre to the top periphery or from the centre to the bottom periphery and, further, occur only for either oil content or iodine value in the same seed, and never the two together in the same seed up to the present. There, however, does not seem to exist any connexion between the patterns of variation of oil content on the one hand and iodine values on the other.

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SUBSTANTIVITY OF PYRETHRINS I AND II TO CATTLE SKIN AND HAIR: BEHAVIOUR OF PYRETHRINS IN A CATTLE SPRAY RACE

By A. A. GOLDBERG and H. J. SMITH

Laboratory experiments have shown that the pyrethrins are substantive to cattle skin and hair. When cattle are passed through a spray race, the concentration of pyrethrins in the circulation liquor decreases. The adsorption rate constant has been calculated for standard conditions and an equation derived for calculation of the concentration after passage of cattle through the spray race. The use of this equation in veterinary practice is discussed.

Introduction

Aqueous emulsions of pyrethrum extract have been extensively used in East Africa as cattle sprays for controlling infestations of the blue tick—*Boophilus decoloratus*.[1]

The comparatively high cost of pyrethrum extract made it necessary to determine the minimum concentration of pyrethrins which gives effective control, that is, the LC_{100}. Accordingly, in a number of experimental sprayings, the pyrethrin content of the liquor was determined at intervals during the period in which cattle passed through the spray race. It was found that a progressive depletion of pyrethrins from the liquor occurred with the passage of cattle. Laboratory experiments with cowhide confirmed this substantivity of the pyrethrins for skin and hair. This observation has an important bearing upon the procedure to be adopted in order to obtain the best results in veterinary practice.

Experimental

Analysis of pyrethrin emulsions

The method of analysis selected was that in which the pyrethrin dinitrophenylhydrazones are separated by chromatography on an alumina column. This procedure accurately records the true pyrethrins—as opposed to the biologically inactive polymerised and hydrolysed pyrethrins.

An aliquot of the emulsion (250 c.c.; 0·01% pyrethrins) was saturated with sodium chloride and extracted with isohexane (7 portions of 70 c.c.). The combined isohexane extracts were distilled almost to dryness and the residue of pyrethrins treated by the method developed by

Smith[2] with the modification that sodium chloride was present during all the extraction procedures in order to break emulsions. It was found that this method gave the correct results for emulsions of known pyrethrin content; the presence of urine, faeces and extracts of soil does not interfere.

Materials

Two emulsifiable concentrates were used. These both contained a non-ionic emulsifying agent octylphenoxypolyethoxyethanol (Ethylan BCP; Lankro Chemicals, Eccles, Manchester); the second emulsion contained, in addition, an anionic wetting agent, triethanolamine lauryl sulphate (Empical TA; Marchon Products, Whitehaven, Cumberland) in order to increase wetting power.

The concentrate compositions were as follows:

(I) Pyrethrum 25% extract (275 c.c.), Ethylan BCP (425 c.c.) and xylene (300 c.c.);
(II) Pyrethrum 25% extract (200 c.c.), Ethylan BCP (250 c.c.), Empical TA (250 c.c.) and xylene (300 c.c.).

Field experiments

The requisite amount of the emulsifiable concentrate was added to a measured volume of water in the spray race tank (1000–3000 litres) and the solution stirred until homogeneous. An initial sample was taken and then further samples removed after the passage of a known number of cattle.

A control experiment was conducted in the absence of cattle when the spray race was run for $\frac{3}{4}$ h. The following results with formulation I show that the losses of pyrethrins during spraying of cattle are not due to aerial oxidation or solar degradation of the pyrethrins.

Time sample taken, min.:	0	10	30	40
Total pyrethrins found: (mg./250 c.c.)	26·68	27·22	26·73	26·57

In a spray race, the liquor from the tank is continuously pumped through a large number of nozzles (18–30) positioned in such directions that cattle walking through the 'tunnel' are wetted over the whole body. The run-off liquor drains back into the tank. A small volume (~0·75 gal.; 3·5 l.) of the solution is removed by each wetted animal; this causes a slow decrease in the volume of the liquor circulating in the tank and spray race.

Theoretical treatment

Decrease in the pyrethrins content of the circulating liquor by adsorption of the pyrethrins on the skin and hair takes place progressively. The adsorption of compounds from solution on to the surface of solid adsorbents has not yet received satisfactory theoretical treatment. Adsorption from solution does not in general lead to layers of adsorbed phase more than one molecule thick, these being held by van der Waals forces to the structure of the substrate. For many such cases the experimental data can be well represented by the Freundlich equation $x/m = kc.1/n$ where x and m are the masses of substance adsorbed and of adsorbent respectively, c is the concentration of the solution when equilibrium is reached and n is an empirical constant usually greater than unity. In the subject under consideration, however, equilibrium between the dispersed pyrethrins in the spray liquor and the adsorbed phase on the skin and hair will not be reached.

For the theoretical treatment it can be assumed that the Mass Action Law applies and that the molar amount of pyrethrins adsorbed by a given area of skin and hair per given unit of time is directly proportional to the concentration of pyrethrins in the spray liquor. Let the initial volume of liquor in the tank be V litres, containing P moles of pyrethrins; the initial concentration of pyrethrins is $c_0 = P/V$ moles/l.

Let the carry-out of liquor from the spray race by simple wetting of the animals be b litres/animal unit. When N animal units have passed through, the volume is depleted by Nb litres and the remaining volume is $(V - Nb)$ litres.

When N animal units have passed through, suppose that, due to adsorption on the cattle skin and hair, the concentration of pyrethrins in the liquor has fallen to c moles/l. Assuming

that the number of moles of pyrethrins dP adsorbed per infinitesimal animal unit dN in the time it is in contact with the spray is proportional to the pyrethrins concentration of the spray liquor, then

$$dP = kc\,dN = -(V - Nb)\,dc \quad . \quad . \quad . \quad . \quad . \quad (1)$$

or $$dN/(V - Nb) = -dc/kc$$

Integration gives

$$-\frac{1}{b}.\ln (V - Nb) = -\frac{1}{k}.\ln c + K$$

where K is the constant of integration.

When $N = 0$, $c = c_0$, hence

$$K = \frac{1}{k}.\ln c_0 - \frac{1}{b}.\ln V$$

$$\therefore\ k = \ln \frac{c_0}{c} \Big/ -\frac{1}{b}.\ln \left(1 - \frac{Nb}{V}\right) \quad . \quad . \quad . \quad . \quad . \quad (2)$$

Expansion of the denominator of equation (2) by Taylor's Theorem gives

$$-\frac{1}{b}\left(-\frac{Nb}{V} - \frac{N^2b^2}{V^2} - \frac{N^3b^3}{V^3} + \ldots\right)$$

$$= \frac{N}{V} + \frac{N^2b}{V^2} + \frac{N^3b^2}{V^3} - \ldots .$$

$= N/V$ to a first-order approximation, because all terms after the first are negligible, since b is of the order of 3 l. and V of the order of 1000–3000 l.

Hence equation (2) becomes

$$k = \frac{V}{N} \ln \frac{c_0}{c} = 2{\cdot}3\frac{V}{N} \log \frac{c_0}{c} \quad . \quad . \quad . \quad . \quad . \quad (3)$$

whence $$c = c_0 \exp(-kN/V) \quad . \quad . \quad . \quad . \quad . \quad . \quad (3A)$$

Equation (3) gives the adsorption reaction constant in terms of the initial pyrethrin concentration and the concentration after passage of N animal units through a spray race having an initial volume V of spray liquor.

Equation (3) may be rewritten

$$\log c = \log c_0 - kN/2{\cdot}3V$$

which implies that if the log (residual pyrethrin concentration) is plotted against the number of animal units N which have passed through the spray race, a straight-line is obtained with intercept

$$\log c_0 \text{ and slope } \tan^{-1}(-k/2{\cdot}3V) \quad . \quad . \quad . \quad . \quad . \quad (4)$$

It is to be noted that the slope is a function of V, the initial volume of liquor in the tank. The larger is V, the less is the slope; that is, the lower the rate at which the spray liquor is depleted of pyrethrins.

The half-life of the pyrethrins in the spray liquor may be related to the number of animals N_h passing through the spray race which effects reduction of the pyrethrin concentration to one half of the initial concentration. From equation (3), when $N = N_h$, $c = c_0/2$ and $N_h = 0{\cdot}69V/k$. The half-life of the pyrethrins is directly proportional to the initial volume of the spray liquor in the tank.

Table I (and Fig. 1, curve A) show the results obtained on passage of 171 cattle through a spray race, the initial volume of liquor in the tank being 900 l. (200 gal.); in this test formulation No. II was used, diluted to give an initial concentration of 0·01% of pyrethrins.

Table II (and Fig. 1, curve B) shows the depletion of pyrethrins occurring with a larger herd of cattle in a different spray race, the initial volume of liquor being 2700 l. (600 gal.) prepared from formulation No. I. The value of the adsorption reaction constant k, calculated from equation (3), is recorded.

Table I

Depletion of pyrethrins in spray race (400 l.) during passage of 171 cattle

No. of cattle passed	Pyrethrin I, mg./250 c.c.	Pyrethrin II, mg./250 c.c.	Total pyrethrins, mg./250 c.c.	k
0	12·88	11·03	23·91	—
	12·50	10·87	23·37	
44	10·63	8·97	19·60	3·8
	11·00	8·69	19·69	
83	8·68	7·86	16·54	3·7
	8·77	8·32	17·09	
124	7·44	7·04	14·48	3·5
171	6·97	6·21	13·18	3·1
	6·91	6·07	12·98	

The constancy of the values found for the adsorption reaction constant k in the experiments carried out in two different spray races on different herds is good; some decrease in the value of k during the course of the spraying would be expected because of the fouling of the wash. The agreement between the experimentally determined half-lives and those calculated from equation (4) is also satisfactory.

Formulation	Experimental half-life (from Fig. 1)	Calculated half-life $N_h = 0{\cdot}69V/k$	Initial volume of liquor
II	174 cattle	183 cattle	900 l.
I	621	600	2700

(Compare the depletion of aldrin and dieldrin from sheep dips.[3])

Table II

Depletion of pyrethrins in spray race (2700 l.) during passage of 500 cattle

No. of cattle passed	Pyrethrin I, mg./250 c.c.	Pyrethrin II, mg./250 c.c.	Total pyrethrins, mg./250 c.c.	k
0	13·2	10·8	24·0	—
100	11·3	10·0	21·3	3·45
150	10·5	9·4	19·9	3·35
200	10·2	8·9	19·1	3·1
300	9·1	7·9	17·0	3·1
350	8·6	7·3	15·9	3·2
400	7·9	7·2	15·1	3·1
450	7·6	6·9	14·5	3·0
500	7·2	6·5	13·7	3·0

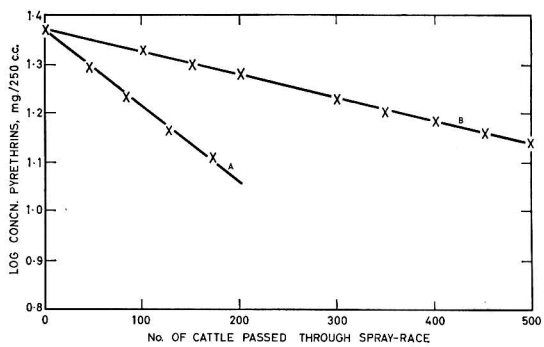

FIG. 1.—*Depletion of pyrethrins from cattle spray race liquors*

A initial volume of liquor in tank 200 gal.
B " " " " " " 600 gal.

Laboratory experiments

A fresh cowhide was washed with water, sundried and cut into pieces (24 in. × 22 in.). These pieces were consecutively immersed for 30 min. each in a stirred pyrethrin emulsion (0·01% pyrethrins; 6500 c.c.; formulation No. II) in such a manner that only the outer surface of the hide, i.e., skin and hair, of area 0·75 sq. ft. came into contact with the liquor. After the immersion of each piece of hide, aliquots (250 c.c.) were removed for analysis. The results are summarised in Table III.

Table III

Adsorption of pyrethrins by pieces of cattle hide

No. of hides immersed before sample taken	Pyrethrins in solution, mg./250 c.c.	k
0	24·08	—
2	20·58	0·51
4	18·20	0·46
6	16·03	0·44
8	13·57	0·47

The adsorption reaction constant is defined by equation (1) : the value of k for the laboratory experiment with pieces of cattle hide is different from the value of k for the field experiments with cattle since different units of area of skin and hair and of time of immersion are employed.

Recovery of pyrethrins adsorbed on cattle hide

The pieces of cattle hide, after immersion in the 0·01% pyrethrin solution for the stated time, were removed, allowed to drain, reweighed and then allowed to dry in the absence of sunlight. From the initial and final concentrations in the bath it is possible to calculate the amount of pyrethrins adsorbed upon the skin and hair; to this must be added the amount of pyrethrins contained in the liquor which wets the hide after it has drained; this latter was calculated to be ~2 mg. of pyrethrins in each case. The pieces of hide were then immersed for 1 h. in stirred (*a*) 0·5% aqueous solution of Empicol TA or (*b*) ethanol. In the first case the aqueous liquor was extracted with isohexane, and in the second case the ethanol was distilled nearly to dryness, in order to determine the extracted pyrethrins. The results are summarised in Table IV.

Table IV

Calculated pyrethrins adsorbed on hide, mg.	Pyrethrins extracted by solvent	% Adsorbed pyrethrins extracted
31	1 mg. (*a*)	3
32	23 mg. (*b*)	72
35	16 mg. (*b*)	46
33	15 mg. (*b*)	45
51	31 mg. (*b*)	61

It is evident that the adsorbed pyrethrin phase is fast to dilute aqueous emulsifying agents but some 40–70% of it is removed by alcohol. (Compare the adsorption of aldrin and dieldrin from colloidal solutions by sheep fleece.[4, 5])

Conclusions

It has been shown that the pyrethrins are substantive to cattle skin and hair and that, when cattle are passed through a spray race, the concentration of pyrethrins in the spray liquor is progressively depleted. If this fact is not borne in mind, it may well happen that, by the time the last part of the herd is passed through the spray race, the pyrethrins concentration may have fallen below the tick death threshold value (LC_{100}).

From the equations developed, the adsorption reaction constant for a given formulation of pyrethrins may be calculated; from this it is possible to calculate the slope of the regression

line of the pyrethrin concentration relative to the number of cattle which have passed through the spray race and, therefore, the residual concentration after the passage of a given number of animals.

The application of these results in veterinary practice is obvious. The blue tick death threshold concentration (LC_{100}) is not known with certainty and probably varies to some extent with climatic and geographical conditions. From the work of Larkin[1] in East Africa it would appear to be of the order of 0·008% pyrethrins in a suitable formulation. Assuming this figure, and taking the value of the adsorption constant k to be 3·2, then the following would be the procedure for treating a herd of 250 cattle:

Pyrethrin 5% w/v (PBK) emulsifiable concentrate (5 pints; 2840 c.c.) is added to water (250 gal.; 1135 l.) in the spray race tank to prepare a 0·0125% (PBK) pyrethrin solution (this equals 0·01% pyrethrins DNP since pyrethrum extract 25% PBK analyses at 22·3% by the A.O.A.C. method and 20·0% by the DNP method).

From equation (3) it follows that, after the passage of 125 cattle through the spray race, the concentration of the liquor will have fallen to 0·0088%. The amount of liquor remaining in the tank will be ~155 gal.; addition of a further 0·92 pints (18·4 fl. oz.; 522 c.c.) of the 5% concentrate will increase the pyrethrins concentration back to 0·0125%. After the passage of a further 75 cattle the concentration will have fallen to 0·0089% leaving a volume of ~100 gal. in the spray race tank. Addition of a further 0·6 pints (12 fl. oz.; 336 c.c.) of the 5% concentrate will bring back the concentration again to 0·0125%; about 50 cattle can then be passed through the race leaving a small amount of residual liquor of concentration 0·0088%.

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THE COMPOSITION OF *PACHYRRHIZUS EROSUS* (YAM BEAN) SEED OIL

By J. H. BROADBENT and G. SHONE

The fatty acid composition of the oil has been examined by gas–liquid chromatography and found to contain 26·7% of palmitic, 5·7% of stearic, 33·4% of oleic and 34·2% of linoleic acids. The unsaponifiable matter of the oil, which contains 55% of digitonide-forming sterols, has been separated by thin-layer chromatography into six components, two of which give positive Liebermann–Burchard and one positive Emmerie–Engel tests.

Introduction

Pachyrrhizus erosus (L.) Urban is a herbaceous vine[1] which is indigenous to Latin America but has a widespread distribution in tropical countries. Its most important use to date has been as a food ; its watery tubers, which can weigh up to 5–10 kg., are eaten raw and are reported to be rich in vitamin C.[2]

The seeds from *P. erosus* have long been used in tropical territories as insecticides and fish poisons,[3] and in recent years have been shown to contain rotenoids to the extent of some 0·5–1%[4] and to exhibit marked toxicity to several species of insect.[5, 6] The seeds are reported to contain 20·5–28·4% of oil,[7] the fatty acid composition of which has been calculated from iodine and thiocyanogen values.[8] The fatty acid composition has not previously been examined by gas chromatography.

Experimental and results

The oil was extracted from seeds of Malayan origin with cold light petroleum* and the acid value, saponification value and unsaponifiable content determined by the method of British Standard 684 : 1958. The iodine value was determined by the Wijs method, see Table I.

The oil gave a typical fixed oil infra-red absorption spectrum and contained 0·6% of conjugated diene ($\lambda_{max.}$ 235 mμ). Conjugated triene was not detected.

Table I

Constants of Pachyrrhizus erosus *seed oil*

	This work	Results of Cruz[8]
Acid value, mg. KOH/g.	1·55	2·6
Saponification value, mg. KOH/g.	194	182·9
Iodine value (Wijs, 30 min.)	84	84·1 (Hanus)
Unsaponifiable content, %	1·4	2·2

Methyl esters were prepared from the mixed fatty acids (420 mg.) by refluxing (5 min.) with methanol/boron trifluoride reagent (6·3 ml.) and separated on a 183 × 0·4 cm. chromatographic column packed with 100–120 mesh Celite impregnated with 10% polyethylene glycol adipate operating at 161° with argon as carrier gas (radium source detector at 1250 V). The fatty acid composition, obtained by internal normalisation, is given in Table II.

Table II

Fatty acid composition of Pachyrrhizus erosus *seed oil*

Acid	This work*		Previous results[8] (from iodine and thiocyanogen values)
	E.C.L.†	%	
Palmitic	16·0	26·7	39·4
Stearic	18·0	5·7	
Oleic	18·2	33·4	33·6
Linoleic	18·8	34·2	27·0
Linolenic	19·5	trace	—

* Theoretical iodine value for this composition 88
† Equivalent chain length

Attempts were made to separate the isolated unsaponifiable material on thin layers of Kieselgel ' G ' (Merck), Kieselgel ' G ' impregnated with silver nitrate,[9] and aluminium oxide (Fluka) (with an extra 5% plaster of Paris added) with use of various solvent mixtures. The first two adsorbents proved to be ineffective in separating some of the components, but thin layers of aluminium oxide with mixtures of diethyl ether and chloroform as eluents separated three major and three minor components (Table III). Concentrated sulphuric acid was used as the detecting agent but thin-layer chromatograms were also treated with sulphuric acid/acetic anhydride and $\alpha\alpha'$-bipyridyl/ferric chloride reagents. Two of the separated components gave positive Liebermann–Burchard reactions and one a positive Emmerie–Engel reaction[10] for reducing compounds (Table III). This last material was not a tocopherol.

* Extraction by Dr. L. Crombie, King's College, University of London.

Table III

Component unsaponifiables separated on thin layers of aluminium oxide

(Eluent, 5% diethyl ether/chloroform)

Component	R_F	Colour with H_2SO_4	Liebermann-Burchard test	Emmerie-Engel test
1	0·82	yellow→grey	—	pink
2	0·57	yellow	—	—
3 (major)	0·48	yellow→purple	—	—
4 (major)	0·42	orange	purple	—
5 (major)	0·33	purple	red	—
6	0·12	grey	—	—
7	0·00	?		

Digitonide-forming sterols accounted for about 55% of the unsaponifiable material. Thin-layer chromatography, under the conditions specified above, of the material liberated from the digitonides gave three coloured spots after sulphuric acid detection, R_F 0·82 (grey), 0·46 (orange: major), 0·00 (orange: trace).

The oil and unsaponifiable material gave negative results when subjected to the Durham,[11] Goodhue[7] and Rogers-Calamari[7] tests for rotenoids.

Discussion

The fatty acid composition of *Pachyrrhizus erosus* seed oil resembles that of cottonseed oil or the more common vegetable oils, although it contains rather more palmitate and correspondingly less oleate and/or linoleate than the latter (Table IV), and would possibly be suitable for use in the edible oil industry.

Table IV

Fatty acid composition (%) of Pachyrrhizus erosus *seed oil compared with that for cottonseed oil*

	C_{16}	C_{18}	$C_{18:1}$	$C_{18:2}$	$C_{18:3}$
P. erosus (this work)	26·7	5·7	33·4	34·2	trace
Cottonseed[12]	19·6	2·7	24·6	50·4	
	17·2	0·9	44·2	33·9	

(18:1–18:3 indicates 18 carbon atoms with 1–3 unsaturated centres)

The tubers of *P. erosus* are used for edible purposes; the seeds could possibly be used to give an edible grade oil and also insecticidal concentrates. The Chinese considered the yam bean to be one of the most promising insecticides as a substitute for derris[4] and various insecticidal compositions have been formulated incorporating *P. erosus* and pyrethrum extracts for use against flies, mosquitoes, etc.[13] However, if the plant is to be of commercial value, improvements by selection and breeding would be necessary.

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NINHYDRIN-REACTING SUBSTANCES FROM APPLE SPURS

By MARIA BIELINSKA-CZARNECKA

In further investigations of the amino-acids present in spurs of apple trees, seven additional compounds to those previously reported[1] have been identified and others detected.

Spurs of Macoun variety were collected on two occasions :

(1) On 31 October, 1962 : freeze-dried and finely powdered.
(2) On 15 January, 1963 : freshly collected for immediate extraction.

Amino-acid substances present were extracted either by shaking the dried material (20 g.) with 75% (v/v) ethanol (300 ml.) for 24 h., or by homogenising the fresh material in 75% ethanol. The extracts were filtered, applied separately to columns of ZeoKarb-225 (H^+ form) resin packed in 75% ethanol and the amino-acids and amides eluted with 2N-aqueous ammonia in ethanol. After concentration, portions of the eluate were absorbed on a column of Dowex-1 (acetate form) resin and eluted with 2N-acetic acid followed by 2N-hydrochloric acid.

Portions of the eluates from both ZeoKarb-225 and Dowex-1 columns were subjected to paper chromatography, with phenol–water (3 : 1 w/v) as first solvent followed by butan-1-ol–acetic acid–water (90 : 10 : 29 v/v). A solution of ninhydrin in ethanol (0·1%) was used to locate amino-acids, or isatin in acetone (0·2%) to confirm imino-acids. Certain compounds were also co-chromatographed with authentic samples with butan-1-ol saturated with 3N-aqueous ammonia as solvent.

Pipecolic acid, ethanolamine, 4-hydroxymethylproline, methionine sulphoxide, β-alanine, homoserine and tyrosine were identified in addition to compounds found previously.[1] The presence of ethanolamine was confirmed by high-voltage paper electrophoresis at pH 6·5. Methionine sulphoxide may have arisen as an artefact by oxidation of methionine during the extraction and chromatographic procedures. An unidentified compound was present in both the acetic acid and the hydrochloric acid eluates from the Dowex-1 columns, which may have been a glutamyl peptide, since on acid hydrolysis (6N-hydrochloric acid at 100° for 24 h.) glutamic acid was liberated. Lysine was not detected in this study although it was reported in apple stem material.[2]

Volatile ninhydrin-reacting compounds were investigated when powdered spur material (1 g.) was treated as follows :

(*a*) suspended in 1% sodium borate solution (about pH 9) in a Markham still and the mixture steam distilled.
(*b*) treated as in (*a*) except that 40% sodium hydroxide replaced borate solution.

The distillates were passed into 0·1N-hydrochloric acid ; any volatile amines remained as their hydrochlorides after evaporation to dryness.

Portions of the distillates were co-chromatographed in butan-1-ol–acetic acid–water solvent[3] and sprayed with ninhydrin. Trimethylamine, methylamine and ethylamine were tentatively identified in solutions from both treatments, together with a slower running unidentified spot.

Three very slow running spots were also noted in low concentration in the distillate from sodium hydroxide treatment, which were probably artefacts produced by decomposition of other labile compounds.

Acknowledgments

The author is grateful to University College, London, and to Professor D. Lewis for facilities in the Dept. of Botany. Thanks are especially due to Dr. L. Fowden for directing this work in his laboratory. Dr. E. A. Bell, Dept. of Biochemistry, King's College, London, kindly carried out electrophoreses. Mr. J. M. S. Potter of the National Fruit Trials, Faversham, Kent, kindly provided the material and Mrs. U. Dzieciol, Research Institute of Pomology, Skierniewice, helped in the preliminary work.

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ERRATA

(1) In the paper by P. K. Datta, A. C. Frazer, M. Sharratt and H. G. Sammons (*J. Sci. Fd Agric,*. 1962, **13,** 556), on p. 565, line 30, for ' low for normal body constituents ' read ' large for normal body constituents '. The important point is that the use of phosphates on food additives should be considered in relation to the total dietary phosphate load.

(2) In the paper by P. W. Ratcliff and M. J. Follett (*J. Sci. Fd Agric.*, 1963, **14,** 138) the length of the column in Fig. 1 (p. 140) should be 17 cm. (not 7 cm.).

(3) In the paper by Jean F. Melvin and Beulah Simpson (*J. Sci. Fd Agric.*, 1963, **14,** 228), the caption to Fig. 3 (p. 233) should read ' changes in soluble sugars during drying '.

(4) In the paper by J. K. R. Gasser and R. J. B. Williams (*J. Sci. Fd Agric.*, 1963, **14,** 269), in the Acknowledgments (p. 277), F. U. Widdowson should read F. W. Widdowson, and Dimwoody should read Dunwoody.

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JULY, 1963

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

JULY, 1963

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Clay mineralogy. R. E. Grim (*Science*, 1962, **135,** 890—898).—A review. T. G. MORRIS.

Formation of gibbsite as a primary weathering product of acid igneous rocks. J. P. Watson (*Nature, Lond.*, 1962, **196,** 1123—1124).—The transitory occurrence of gibbsite during the early stages of the weathering of granite is confirmed by differential thermal analysis and *X*-ray analysis of material taken at various depths from a granite weathering crust in S. Rhodesia. W. ELSTOW.

Nomenclature of soil horizons of eroded skeletal soils. Z. Gračanin (*Z. PflErnähr. Düng.*, 1963, **101,** 42—48).—Nomenclature for normal and eroded soil profiles is inadequate for eroded skeletal soils. A further horizon—the A_{sk}—is proposed. This horizon protects horizons below it from further erosion. M. LONG.

Undisturbed soil columns for the study of soil genetics and formation mechanisms, using radioactively labelled soil constituents. I. Assay technique and experimental lay-out. H. W. Scharpenseel and W. Kerpen (*Z. PflErnähr. Düng.*, 1963, **101,** 1—10).—The apparatus consists of a series of stainless steel tubes to which are attached columns of undisturbed soil obtained by a special boring device. Pumps, controlled by self-operating switches, are used to supply eluting solvents. The eluted ions are collected on ion-exchange columns. The soil columns are charged with ^{14}C and ^{3}H-labelled humic and fulvic acid prep. and ^{55}Fe-labelled clay mineral and Fe compounds. The feasibility of the radioautography technique for the evaluation of the simulated soil dynamic process is confirmed. M. LONG.

Vegetation and soils of Fishtail Mesa, Arizona. D. A. Jameson, J. A. Williams and E. W. Wilton (*Ecology*, 1962, **43,** 403—410).—Profile characteristics of these soils are considered in relation to the distribution of tree and shrub species. A. G. POLLARD.

Geological features of importance for the productivity of the soils of Norway. J. Låg (*Soil Sci.*, 1963, **95,** 1—8).—The distribution of the different rocks in Norway is discussed. Soil forming processes, principally glacial erosion are also considered. T. G. MORRIS.

Fertility of the lowland soils of British Honduras. D. H. Romney (*Emp. J. exp. Agric.*, 1962, **30,** 95—107).—Soil analyses are presented and discussed in relation to soil formation and natural vegetation. The main agricultural soils are black soils over limestone, red soils over tuffaceous limestone, soils from acid materials, alluvial soils and swamp soils. Results of field trials with a no. of crops are presented, together with foliar analyses. Crops generally responded to P and N but not to K. A. H. CORNFIELD.

Absence of carbon-14 activity in dolomite from Florida Bay. K. S. Deffeyes and E. L. Martin (*Science*, 1962, **136,** 782).—Dolomite crystals extracted from recent carbonate sediments in Florida Bay were shown by ^{14}C analysis to be older than 35,000 years. Recent sedimentation in this area began less than 4000 years ago. The dolomite therefore must be derived from older rocks. Indications are that dolomite is not being formed at present. T. G. MORRIS.

Soil survey method. N. H. Taylor and I. J. Pohlen (*N.Z. Dep. sci. industr. Res. Soil Bur. Bull.*, 1962, No. 25, 242 pp.).—A compilation of modern methods of soil examination and description as required for comprehensive soil surveys. Sections included cover pedological terminology; essential observations for the description of the soil site; the profile characteristics (illustrated) leading to soil mapping and classification; measurement of soil radioactivity; hydrological characteristics; preservation of soil monoliths; pasture examination and the presentation of records. Soils of N.Z. are described and classified on the basis of the system shown. A. G. POLLARD.

Mechanics of water movement and storage in soils: a teaching technique. J. A. Hobbs and L. E. Wittsell (*Agron. J.*, 1963, **55,** 67—70).—Water movement and storage are described for a tank equipped with a porous cover and three storage sections. Empirical equations developed by hydraulic engineers to account for water flow are employed. Either graphic or integration methods may be used to determine how the velocity of water flow and the rate of water storage or loss change with time. Analogies are drawn between water movement and storage in the tank and water movement and storage in the soil. A. H. CORNFIELD.

Application of the vacuum oven to moisture determinations in biological materials. J. F. Connolly and J. O'Shea (*Irish J. agric. Res.*, 1962, **1,** 334—338).—To ensure the drying of a range of agricultural materials in a vac. oven within 4 h. the optimum conditions were 85° and 25 in. vac. A. G. POLLARD.

Gaussian elimination technique for solving the diffusion equation for moisture movement in unsaturated soil. G. L. Ashcroft (*Dissert. Abstr.*, 1962, **23,** 1480—1481).—The diffusion equation has been successfully used to describe moisture movement in soil. Success depends on the Boltzmann transformation which can be used only for semi-infinite, uniform regions. Solutions obtained by the Gaussian elimination technique were compared with solutions obtained by the Boltzmann transform technique. Both systems give similar results for horizontal flow in semi-infinite, uniform media. Solutions were also obtained for layered soils. These solutions fit the experimental data almost as well as do the solutions for uniform soil. F. C. SUTTON.

Effect of isotropic confining pressure on hydrostatic pressure of water in unsaturated soil. P. R. Day and W. M. Forsythe (*Science*, 1962, **136,** 779—780).—A theory is presented for the response of the pore water pressure to pressure applied isotropically to unsaturated soil. Results show that the response is determined by two shrinkage characteristics of the soil. A mathematical representation of the theory is given. T. G. MORRIS.

Radial-flow cell for soil-water measurements. L. A. Richards and P. L. Richards (*Proc. Soil Sci. Soc. Amer.*, 1962, **26,** 515—518).—A radial-flow type pressure cell is described for bringing soil cores to known matric suction values. A mathematical analysis is given for calculating the soil-water diffusivity and conductivity from the rate of water transfer during approach to equilibrium. A. H. CORNFIELD.

Calculated potential evapotranspiration and soil-moisture deficits for North County Dublin. W. Burke (*Irish J. agric. Res.*, 1962, **1,** 329—333).—Values for the potential evapotranspiration (P.E.) for the months April—Dec., calculated from meteorological data. The soil-moisture deficit determined as the difference between P.E. and rainfall exceeded the equivalent of 4 in. of rain in 10 of 12 years and exceeded 8·5 in. in one season. A. G. POLLARD.

Effects of compaction on physical properties of sand–soil–peat mixtures at various moisture contents. W. E. Swartz and L. T. Kardos (*Agron. J.*, 1963, **55,** 7—10).—The effects of compaction on water percolation rate and aeration porosity of soil–sand–peat mixtures varied with the proportion of the three constituents and also with the type of soil. The textural fraction 0·25—2·00 mm. was the dominant fraction controlling percolation rate. At high moisture contents compaction resulted in inadequate percolation rates if the mixture contained <50% sand. In mixtures containing 70% of sand moisture retention was usually poor. A. H. CORNFIELD.

Soil layering and compaction effects on unsaturated moisture movement. J. R. Eagleman and V. C. Jamison (*Proc. Soil Sci. Soc. Amer.*, 1962, **26,** 519—522).—Hydraulic conductivity of the junction between soil layers differing in texture, structure and compaction, as obtained from a laboratory model, were different in magnitude depending on the direction of water movement with respect to pore size. Soil moisture was transferred much more rapidly from the large to the small pore spaces than in the reverse direction. A. H. CORNFIELD.

Specific surface determination of expansible layer silicates. M. H. Milford and M. L. Jackson (*Science*, 1962, **135,** 929—930).—A vac. oven technique using glycerol at 130° is described for determining quant. the amount of glycerol sorbed by clay minerals, the vapour pressure of the glycerol within the oven being controlled by temp. adjustments. Montmorillonite absorbed a monolayer of glycerol having liquid-glycerol *d*, thus providing quant. sp. surface analysis; vermiculite adsorbed a monolayer of lower *d* than that of liquid glycerol. T. G. MORRIS.

Formation of double hydroxides and the titration of clays. R. C. Turner and J. E. Brydon (*Science*, 1962, **136,** 1052—1053).—Wyoming bentonite saturated with either Al, Fe, La or Mg was

suspended in 10^{-3}M-$MgCl_2$ solution and then titrated with MgO at 25°. After each increment of MgO had been added the suspensions were stirred for 24 h. if the pH was less than 7 and for 48 h. if higher than 7, with CO_2-free air bubbling through. Buffering occurred at pH 8·7 and 9·2 respectively for clays saturated with Al and Fe, and at pH 10·0 for those saturated with La and Mg. The formation of $Mg(OH)_2$ should occur at pH 10 indicating that the buffering of Mg and La clays was due to pptn. of $Mg(OH)_2$ but this reaction does not account for the behaviour of the Al and Fe clays. *X*-ray examination showed that the ppt. with Al and Fe clays was the double hydroxide of Mg and Al or Fe. T. G. MORRIS.

Influence of exchangeable ions on release of mineral-bound ions. L. Wiklander and E. Koutler-Andersson (*Soil Sci.*, 1963, **95,** 9—15).—Soils in which the clays contained principally illite with differing amounts of kaolinite, montmorillonite, vermiculite, feldspar and quartz were treated with acetates of H, Na, K, NH_4, Mg or Ca, washed thoroughly and then kept moist at either 2—4° or 20° under aerobic conditions. The content of exchangeable cations was determined after 1—2 years. Temp. of storage had no marked effect. Irrespective of the kind of saturating ion the release of ions from non-exchangeable to exchangeable took place mainly in the first year of storage. The exchangeable ions exerted a specific effect on the transfer of other ions from lattice-bound to exchangeable or sol. forms. Thus, H^+ strongly promoted the release of Mg, compared with NH_4^+, K^+ or Na^+; NH_4^+ reduced the release of K^+ but it increased substantially that of Ca^{2+}. In the Mg^{2+}-saturated soils no Ca^{2+} was transformed during the 2 years in any soil. Saturation with Mg^{2+} and Ca^{2+} reduced the total mobilisation of cations. At low pH levels in the H series much mobilisation of Mn and P occurred. Differential thermal analysis of the clay material indicated that, in the H^+-saturated soils there were changes over the range 20—2000° inconsistent solely with the substitution of H, but due to lattice alterations caused by transfer of Al^{3+}, Fe^{3+} and other ions from lattice positions to sol. forms. T. G. MORRIS.

Isotopic exchange in soil fertility studies. C. G. Lamm, E. H. Hansen and J. A. Jørgensen (*Soil Sci.*, 1963, **95,** 16—23).—Natural Ca apatite and synthetic Ca phytate were mechanically stirred with water containing NaN_3 for 24 h. after which ^{32}P was added and β-activity was determined at intervals. Isotopic exchange with the Ca apatite reached a steady state in 2—3 days; that with the phytate was reached in a few h. and involved more P than in the case of the apatite. Isotopic exchange between equilibrium solutions containing 9·8 mg. of Mn per litre at pH 6·8 and Mn phosphate reached 100% of the total Mn content of the solid phase in 20 h., but with Mn oxide at pH 7·8 only 20% of the total Mn was involved in 5 days. Increased rates of stirring and increasing the solid/liquid ratio in the solution increased the rate of exchange. Mn phosphate was a better source of Mn than the oxide. When Mn phytate was equilibrated with water for 12 days at room temp. and then either ^{32}P or ^{54}Mn was added, exchange to most of the Mn in the solid phase but only to 1—2% of the P occurred in 140 h. T. G. MORRIS.

Effect of fertilisers, sulphur and mulch on East African tea soils. II. Effect on the base status and organic matter content of the soil. A. N. Smith (*E. Afr. agric. For. J.*, 1962, **28,** 16—21).—Application of $(NH_4)_2SO_4$ (N 40 lb./acre) over 4 years decreased exchangeable Ca, K and Mg in these soils. Urea (N 40 lb./acre) increased exchangeable K and org. matter. Application of S decreased exchangeable Ca, K and Mg, decreased org. matter in one soil and increased it in another. Mulch increased exchangeable Ca, K and Mg. A. H. CORNFIELD.

Removal of salts from saline-alkali soils by use of porous ducts. R. S. Chahal (*J. Indian Soc. Soil Sci.*, 1962, **10,** 289—293).—Salts may be removed from apparently impermeable soils by digging holes and filling these with porous material prior to irrigation. Ducts filled with glass beads were formed in columns of soil which were leached with saturated solutions of gypsum. High concn. of salts could move through soil under steep concn. gradients by simple diffusion. A. G. POLLARD.

Maintenance of nitrogen in a Brunizem soil of Eastern Nebraska. D. H. Sander, L. Chesnin, H. F. Rhoades, D. P. McGill, W. L. Colville and W. E. Lyness (*Agron. J.*, 1963, **55,** 53—55).—All rotations, except maize–oats–wheat without added N, maintained soil-N over 12 years on a Brunizem (silty clay loam). Rotations with legumes were more effective than were those with bromegrass. Application of 40 lb. of inorg. N per annum resulted in significantly greater levels of soil-N during bromegrass and grain rotations, but these levels fell to that of no-N plots after 5 years. A. H. CORNFIELD.

Available nitrogen in soils. V. Iswaran, Abhishwar Sen and A. K. Rishi (*Indian J. appl. Chem.*, 1962, **25,** 47—48).—Glucose decomposition values (**I**) of various unsterilised soils were correlated with total N, mineral N and N obtained by alkaline and acid $KMnO_4$ oxidation. The results showed correlation between **I** and mineral N to be significant at the 1% level of confidence and that between **I** and N obtained by acid $KMnO_4$ oxidation to be significant at the 5% level. No significant correlation was obtained between **I** and N obtained by alkaline $KMnO_4$ oxidation. Probably N values obtained by acid $KMnO_4$ oxidation are nearer to the available N figure than are those obtained by alkaline oxidation. M. O'LEARY.

Effect of winter rainfall on the amount of nitrogen available to crops. F. van der Paauw (*Plant & Soil*, 1962, **16,** 361—380).—Grain yields of rye grown without added N were negatively correlated with rainfall during the previous winter (Nov.—Feb.). Where N was applied winter rainfall had little effect on yields. Similar, though not so pronounced, effects occurred with potatoes. Interception of winter rainfall and analysis of various soil layers for sol. N compounds indicated that differences in yields due to winter rainfall were due to differences in the extent of leaching of sol. N compounds and their distribution in the subsoil. A. H. CORNFIELD.

Effect of salinity on the transformation of nitrogen in the brackish water fish-farm soils. L. N. Mandal (*J. Indian Soc. Soil Sci.*, 1962, **10,** 255—261).—When no fertilisers were used the available N (NH_3, NO_3^-, NO_2^-) content of the pond water increased with salinity to a max. with 20 pt./1000 salinity. In the underlying soil the available N (KCl extract) decreased to a min. with salinity ≏20 and increased again with greater salinity. Where N fertilisers [urea, $(NH_4)_2SO_4$] were applied N availability in soil and water increased with salinity, N losses were reduced, absorption of N by the soil complex increased and rates of nitrification diminished. A. G. POLLARD.

Soil nitrogen. VII. Correlations between measurements of nitrogen status of soils and nitrogen % and nitrogen content of crops. J. K. R. Gasser and R. J. B. Williams (*J. Sci. Fd Agric.*, 1963, **14,** 269—277; cf. J.S.F.A. Abstr., 1961, ii, 251).—Laboratory measurements of 'available' soil-N were used to assess its effect on dry matter, N content, and N % of ryegrass grown in pots, of barley and wheat seedlings grown in boxes and of barley grain from field crops grown on soils without fertiliser-N. Mineral-N in fresh soil and the increase in mineral-N after incubating re-wetted air-dry soil (**I**) were all significantly correlated with yields and N contents of ryegrass, barley and wheat seedlings and barley grain. These soil-N measurements were also significantly correlated with N % in barley grain and sometimes with N in ryegrass and in barley and wheat seedlings. **I** was significantly correlated most often. Soil-N measurements were correlated better with N contents of crops than with yields of dry matter. E. M. J.

Sorption of ammonia in a dry system in relation to the chemical properties of clays and soils. D. W. James (*Dissert. Abstr.*, 1962, **23,** 391).—Ammonia was chemisorbed by the exchangeable cations on 2 : 1 and 1 : 1 type clays. The retention process in all clays was augmented by the sorption of NH_3 to hydroxyl groups which have weak acid properties; this appeared of much more importance in the kaolinite systems than in the other clays. Under suitable R.H. conditions sorbed NH_3 may be replaced by water. Most of the NH_3 sorbed by natural soils was a result of chemisorption to polar groups in the humus. F. C. SUTTON.

Distribution and transformations of soil phosphorus on differentially treated plots at the Aledo soil experimental field. M. K. John (*Dissert. Abstr.*, 1962, **23,** 372).—A study was made of the accumulation, movement, distribution and chemical changes in soil P in the plots of a long-term fertiliser trial after 51 years of cropping and differential fertilisation. Evidence obtained indicated that all the applied P remained in the 0—9-in. depth. F. C. SUTTON.

Influence of phosphorus source and soil moisture on the solubility of phosphorus. N. A. Gough and J. D. Beaton (*J. Sci. Fd Agric.*, 1963, **14,** 224—228).—Two calcareous soils—a stony sandy loam (*M*) and a clay loam (*N*)—were treated with various Ca and NH_4^+ phosphates and maintained at a fairly constant moisture content. After 5 weeks three consecutive crops of lucerne were sown and harvested over a period of 6 months in a growth chamber and then equilibrium soil solutions were obtained from both the soils. $Ca(H_2PO_4)_2$ ($\frac{1}{2}$pCa + pH_2PO_4) (**I**) and lime (pH − $\frac{1}{2}$pCa) potentials and $CaHPO_4$ potentials ($pCaHPO_4$) (**II**) were calculated from pH, Ca and P measurements in these solutions. A plot of the lime and phosphate potentials on a solubility diagram showed that all the experimental values were near or above the solubility curve for $CaHPO_4.2H_2O$. Phosphate potentials of the *M* soil were lower than those of the *N* soil. The moisture regimes tested did not affect the lime or phosphate potentials in either soil. The **II** of hydroxyapatite was significantly higher than for all the other sources on the *M* soil and also that of $NH_4H_2PO_4$ and **I** on the *N* soil. The

lowest **II** occurred with the *N* soil. Moisture did not influence **II** on either soil. (21 references.) E. M. J.

Organic phosphorus in Finnish soils. A. Kaila (*Soil Sci.*, 1963, **95,** 38—44).—Samples from the plough layer of many cultivated mineral soils and from deeper layers, together with cultivated humus and virgin soils were analysed for total P, org. P, Al, Fe and pH level. Org. P contents ranged from 100 to 940 p.p.m. in the cultivated surface soils, values in the clay soils being higher than in the loam and silt soils. The org. P content was 17—68% of the total P in the cultivated soils. P in deeper layers was generally low. No close correlation was found between the C content and the org. P content. T. G. MORRIS.

Forms of phosphorus and forest types in the Duke Forest [N. Carolina]. Choon M. Kim (*Ecology*, 1962, **43,** 535—538).—In seven different forest types the total, org. and available P diminished with increasing depth of soil to reach a min. in the C horizon. Differences between types in the vertical distribution of the three P forms were too small to constitute a factor limiting forest types. Probably forest litter has an important influence in supplying P to growing plants. A. G. POLLARD.

Separation of aluminium phosphate from iron phosphate in soils. S. C. Chang and F. H. Liaw (*Science*, 1962, **136,** 386).—Extraction of soil with alkaline 0·5N-NH_4F for periods up to 24 h. removed more P than did extraction for 1 h. with neutral 0·5N-NH_4F, but the increased amount of P was proportional to the amount of Fe phosphate present. T. G. MORRIS.

Determination of total phosphorus [in fertilisers] by the quinoline molybdate method. W. M. Hoffman (*J. Ass. off. agric. Chem., Wash.*, 1962, **45,** 999—1003).—Collaborative results obtained by the quinoline molybdate method were precise, and more accurate but lower than those obtained by the official (A.O.A.C.) volumetric method. There was no significant difference between the results obtained by the volumetric and gravimetric procedures in this method. A. A. ELDRIDGE.

Automatic photometric determination of phosphorus [in fertilisers] as molybdovanadophosphoric acid. R. J. Ferretti and W. M. Hoffman (*J. Ass. off. agric. Chem., Wash.*, 1962, **45,** 993—996).—Determinations based on the formation of molybdovanadophosphoric acid are carried out in a Technicon AutoAnalyzer. The flow scheme (providing a water wash between samples) and the flow cuvette are figured. The precision of the method, while acceptable, is not as good as that of the NH_4 molybdate volumetric and quinoline molybdate gravimetric methods. A. A. ELDRIDGE.

Potassium status of Sudan clay soils. A. Finck (*Plant & Soil*, 1962, **16,** 293—311).—The exchangeable K content of these soils was usually very high (>400 p.p.m.) but reserve K (sol. in boiling N-HNO_3) was not high considering the high clay contents of the soils. Although the more productive areas were highest in both clay content and exchangeable K, the K % in cotton leaves was very similar in all areas and was always above the deficiency level normally reported. The extent of K fixation by the soils was independent of their clay content. Fixation was negligible, and sometimes negative, with low rates of applied K, but increased with rate of application of K, reaching a max. in an exponential-type curve. Cotton on these heavy soils is well supplied with K for the present and the near future. A. H. CORNFIELD.

Soil potassium and the growth of vegetable seedlings. F. Haworth and T. J. Cleaver (*J. Sci. Fd Agric.*, 1963, **14,** 264—268).—The rate of growth and the K content of seedlings of carrot, lettuce and onion was greater on plots which had received applications of farmyard manure (FYM) than on those given only mineral fertilisers including K_2SO_4 (150 lb. of K/acre). The exchangeable K content and the energy of exchange for the replacement of Ca with K (ΔF value) after application of 400 lb. of K/acre were similar to those in soil which had received FYM annually for 8 years. In both cases uptake of K by plants was similar; growth was adversely affected when application of K fertiliser was >200 lb. K/acre. Fixation of added fertiliser-K could explain the relatively poor growth response of crops at Wellesbourne to application of fertiliser-K at ~100 lb. K/acre. For vegetable growing the soil K reserves should be kept permanently at a high level. (11 references.) E. M. J.

Effects of potassium and lime on the relation between phosphorus in soil and plant, with particular reference to glasshouse tomatoes, carnations and winter lettuce. G. W. Winsor and M. I. E. Long (*J. Sci. Fd Agric.*, 1963, **14,** 251—259).—The P content of the leaves of glasshouse tomatoes, carnations and winter lettuce was decreased by additions of K_2SO_4 and lime to the soil, except at low levels of soil P. Liming increased the amounts of P extracted by 0·5N-AcOH and by Morgan's solution. In this study liming was generally advantageous to the lettuce crops (eliminating toxic effects of Mn and improving growth), the carnation crop benefited somewhat, but for tomatoes increase of pH to 7·2—7·5 was definitely adverse. The effect of pH on the content of acid-sol. P in the soil is discussed. (15 references.) E. M. J.

Iron, organic matter and other factors limiting primary productivity in a marl lake. C. L. Schelske (*Science*, 1962, **136,** 45).—Primary productivity of phytoplankton measured as the rate of ^{14}C uptake was increased in the water of a marl lake by the addition of Fe and other nutrients. When no Fe was added with the nutrients the productivity was not affected. If the Fe was added as chelate the productivity again increased. The results are discussed. T. G. MORRIS.

Soil analysis and the assessment of fertility in tropical soils. P. H. Nye (*J. Sci. Fd Agric.*, 1963, **14,** 277—280).—A brief review in which some comparisons between temperate and tropical soils are made. The following are covered: importance of reserves of rapidly weatherable minerals and of org. matter, proportions of nutrient cations, usefulness of pH and soil testing. (14 references.) E. M. J.

Soil and plant manganese. I. Manganese in soil and its uptake by oats. E. R. Page, E. K. Schofield-Palmer and A. J. McGregor. **II. Relationship of soil pH to manganese availability.** E. R. Page (*Plant & Soil*, 1962, **16,** 238—246, 247—257).—I. The uptake of Mn by oats was significantly correlated with water-sol. soil Mn at each of 8 locations. Regression equations relating plant Mn with water-sol. soil Mn are presented for each location. The constant term in the regression equation was roughly proportional to total soil Mn.

II. Results from field tests showed that the relation of soil pH to water-sol. Mn was found to be incompatible with the theory that non-availability of Mn is the result of the formation of insol. higher oxides of Mn at high pH values. In laboratory tests increasing soil pH resulted in reduction in water-sol. Mn content within 2 h., indicating that biological oxidation is not responsible for reduced availability. The relationship between pH and −log water-sol. Mn (pMn) was curvilinear, whereas the formation of higher oxides would imply a linear relationship. Reduced Mn availability with increasing pH may be due to formation of complexes between Mn and soil org. matter. A. H. CORNFIELD.

Aluminium in soils and plants on the coastlands of British Guiana. S. A. Harris (*J. Sci. Fd Agric.*, 1963, **14,** 259—263).—Extractable Al in the soils (0·5N-AcOH) depends on the org. matter content, but is not related to the Al contents of the leaves of natural vegetation. The Al contents of leaves varied between species and between plants growing in different soils. The presence in soils of extractable Al in several forms which vary considerably in their rates of absorption by and mobility within plants is indicated. (15 references.) E. M. J.

Mechanical and chemical analysis of agricultural limestone as affected by type of sieving. P. Chichilo and C. W. Whittaker (*J. Ass. off. agric. Chem., Wash.*, 1962, **45,** 1004—1010).—Fractions obtained by dry sieving weighed more than those obtained by wet sieving. The wet procedure increased the average cumulative % passing a No. 270 sieve from 15·20 to 30·19%. Some samples that fail to meet the required specifications by the dry method would pass by the wet method. Fractions obtained by wet sieving had a higher total neutralising power than had the corresponding dry-sieved fractions. A. A. ELDRIDGE.

Determination of niobium in pyrochlore soils. J. M. Bakes, G. R. E. C. Gregory and P. G. Jeffery (*Anal. chim. Acta*, 1962, **27,** 540—544).—Nb is extracted from the soil sample by heating with conc. HF, and the extract is evaporated with H_2SO_4 to remove SiO_2. The Nb is then precipitated with tannin in the presence of cinchonine and hydroxyammonium chloride, the ppt. is fused with $K_2S_2O_7$ and treated with H_2O_2. The residue is dissolved in a mixture of conc. H_3PO_4 and conc. H_2SO_4 and, after the addition of aq. H_2O_2, the optical density is determined at 365 mμ. Losses of Nb are determined by an isotope dilution method. The recovery of Nb averages 96·27% for samples containing 0·55—7·8% of Nb. H. N. S.

Sorption of strontium in Schleswig-Holstein soils. H. v. Reichenbach and G. v. d. Bussche (*Z. PflErnähr. Düng.*, 1963, **101,** 24—33).—Sr is retained in the top few cm. of the soil and its retention is almost independent of the soil properties. It is partly rendered non-exchangeable. This fraction increases with increasing C and humus content and exchange capacity and, slightly, with decreasing clay content and pH of the soil. Since decontamination measures increase the ion concn. of the soil solution (which at the same time reduces the uptake of Sr by the plant) the distribution of Sr tends to become more uniform throughout the soil. M. LONG.

Rainfall and deposition of strontium-90 in Clallam County, Washington. E. Hardy and L. T. Alexander (*Science*, 1962, **136,** 881—882).—Four sites were selected in the county with rainfall varying

from 15 to 110 in. A linear relationship was found between ^{90}Sr deposited and precipitation. Extrapolation to zero rainfall indicated that a significant portion of the ^{90}Sr was not brought down in the rain. T. G. MORRIS.

Influence of liming and mineral fertilisation on plant uptake of radiostrontium from Danish soils. A. J. Andersen (*Soil Sci.*, 1963, **95**, 52—59).—^{89}Sr and ^{90}Sr were added to soils together with fertilisers and crops were grown. The ^{89}Sr content of clover grown in 20 soils averaged about 3 times that of the ryegrass, with definite differences for different soils. The ^{89}Sr content of crops on a sandy soil with low pH and Ca content was 5 to 6 times that on loamy soils of high pH and high Ca content. The ratio $^{89}Sr/Ca$ was identical for both crops on the same soil. In general the ^{89}Sr concn. and the ratio decrease with increasing exchangeable Ca in the soil. Addition of 4 or 8 mequiv. of $CaCO_3$, $MgCO_3$ or $MgSO_4$ per 100 g. of soil caused a temporary lowering of dry-matter production. The ratio $^{89}Sr/Ca$ decreased during the growing season. Increasing levels of $CaCO_3$ decreased ^{89}Sr levels in plants, the effect being more pronounced with $MgCO_3$ or $MgSO_4$. In soils in which oats responded to N the ^{90}Sr content of the crop varied with the soil, being higher on a sandy soil than on a clay. Ample supplies of N reduced the ^{90}Sr levels in the grain, but increased it in the straw. Increasing N levels reduced the $^{90}Sr/Ca$ ratio. Heavy applications of K reduced the ^{90}Sr uptake. T. G. MORRIS.

Disposal of large amounts of Ferrosul, a steel processing by-product, and its influence on soil factors and plant growth. J. L. Stroehlein (*Dissert. Abstr.*, 1962, **23**, 787).—The effects of applying large quantities of Ferrosul [spent pickle liquor from acid pickle bath (2% H_2SO_4 containing iron sulphate) neutralised with lime] on soils and crop growth were studied with emphasis on the problem of maintaining a sufficient supply of available soil P. Ferrosul can be disposed of by making heavy applications to soils. Any problems of P fixation can be alleviated by banding P fertiliser. F. C. SUTTON.

Decomposition of forest litters. III. Changes in carbohydrate constituents. F. J. Sowden and K. C. Ivarson (*Plant & Soil*, 1962, **16**, 389—400).—Initially both coniferous and deciduous litters contained the same types of sugars, although the coniferous litter was higher in arabinose and mannose and lower in xylose content than the deciduous litter. For the first 6 months of decomposition the xylose, arabinose, mannose, glucose and uronic acid contents of the coniferous litter remained about constant, but fell to about half this level after 40 months. Galactose content did not change with time. In the deciduous litter xylose, glucose and uronic acid decreased over the whole 40 months period whilst the other sugars showed a slight decrease in concn. In general, the coniferous litter decomposed more slowly than did the deciduous litter in the first 6 months. A. H. CORNFIELD.

Factorial experiments on forest humus decomposition. P. J. Viro (*Soil Sci.*, 1963, **95**, 24—30).—Samples of air-dry humus from beneath different tree covers were treated with P, N, K and $CaCO_3$, moistened to water capacity, inoculated with a suspension prepared from fresh humus and incubated at 20° and 90% R.H. for 8 months. In all cases the addition of lime reduced the wt. of org. matter. N accelerated and K retarded decomposition in the limed samples. In the controls the mobilised N decreased during the test mainly as a decrease in NH_3-N. In all pots, liming increased NO_3^--N but in unlimed pots the NO_3^- level reflected the amount of exchangeable Ca in the humus used. Added K and P decreased nitrification. Lime and N decreased whereas P and K increased NH_3-N. Only P increased total mobilised N in all pots. Total N increased in contols and lime enhanced this. Addition of K and P decreased the total N. Liming immobilised N considerably. CO_2 evolution was more rapid in all cases at the beginning of the incubation. The effect of lime varied but eventually always depressed the CO_2 evolution. Liming increased the no. of bacteria and actinomycetes but decreased the no. of moulds. P and N increased the no. but only those of moulds to any significant extent. T. G. MORRIS.

Decomposition of ^{14}C-labelled barley straw in soil. H. Sørensen (*Soil Sci.*, 1963, **95**, 45—51).—Straw from barley grown in an atm. containing 100 μc of ^{14}C, and products extracted from it with water and with ethanol, were mixed with loam soil, moistened with water containing $(NH_4)_2SO_4$, and incubated in CO_2-free air at 20° in the dark. Of the C added as hemicellulose 65% was evolved as CO_2, but only 4% of that added as lignin. In most cases the decomposition of the native org. matter was stimulated by the added straw material. The distribution of the ^{14}C after incubation accounted for about 90% of the activity added; it was distributed between fulvic acid, humic acid and humin. T. G. MORRIS.

Effect of waterlogging on fixation of nitrogen by soil incubated with straw. N. J. Barrow and D. S. Jenkinson (*Plant & Soil*, 1962, **16**, 258—262).—When straw was incubated with a calcareous soil under waterlogged conditions overall N_2 fixation occurred if the atm. above the water contained O_2, but not when O_2 was excluded. Moist soil (60% of max. water-holding capacity) failed to fix N_2 in presence or absence of O_2. A. H. CORNFIELD.

Tea waste as a source of organic manure. D. L. Sharma and K. M. Mehta (*J. Indian Soc. Soil Sci.*, 1962, **10**, 277—282).—Analyses (N, P, K, org. C) of waste tea from hotels etc. are recorded. Nitrification of the waste in soil was slow (120 days) but was accelerated somewhat by addition of a slurry of cattle urine and dung. A. G. POLLARD.

Radio-chromatographic investigations into the exchange of sulphate and the sulphur-amino-acids cystine and methionine in soil and humic acids. H. W. Scharpenseel and R. Krausse (*Z. PflErnähr. Düng.*, 1963, **101**, 11—23).—Sulphate, cysteic acid, cysteine sulphinic acid, taurine, methionine sulphoxide, methionine sulphone, cystine and methionine are the main components, resulting from the hydrolysis of soil and humic acid samples treated with SO_4^{2-} (**I**), methionine (**II**) and cystine (**III**), all labelled with ^{35}S. Pptd. humic acids absorb the least amounts of these. The hydrolysates, obtained from plants treated with **I**, **II** and **III** show a strong methionine peak in the absence of **III**. Further elution releases only minor amounts of compounds other than those above. M. LONG.

Microbiology of the nitrogen cycle in Ghana soils. J. Meiklejohn (*Emp. J. exp. Agric.*, 1962, **30**, 115—126).—Both forest soils (high in exchangeable bases, org. matter and N) and grassland soils (less fertile and particularly low in available N) were rich in N-fixing bacteria, with no significant differences in no. between the two types. While the cover was maintained, forest soils contained many NH_4^+ and NO_2^--oxidising bacteria, whereas grassland soils contained few NH_4^+ and very few or no NO_2^- oxidisers. None of the grassland soil samples taken after the start of the rainy season contained any NO_2^- oxidisers. The lack of available N in Ghana grassland soils is due mainly to absence of NO_2^- oxidisers. A. H. CORNFIELD.

***Azotobacter* in paddy soil.** G. Rangaswami and K. T. Subbaraja (*Proc. Indian Acad. Sci.*, 1962, **56B**, 174—183).—In paddy soil undergoing seasonal variations of waterlogging and drying, the *Azotobacter* content varies accordingly, max. occurring in dry soil. *A. chroöcoccum* was the most efficient and with *A. agilis*, *A. beijerinckii* and *A. vinelandii* fixed max. N at pH 6·0—7·0 of the substrate whereas *A. indicus* and *A. lacticogenus* were more active in the range pH 3·0—8·0. (21 references.) C. A. P.

Rôle of nodule bacteria in nitrogen nutrition of leguminous plants. L. M. Dorosinskiĭ, N. M. Lazareva and V. T. Emtsev (*Mikrobiologiya*, 1962, **31**, 1061—1066).—Lupins were grown in river sand containing two different proportions of NH_4NO_3 and the same amounts of non-nitrogenous plant nutrient. One group was inoculated with active nodule bacteria, and another with inactive bacteria, the control being untreated. Inoculation with active nodule bacteria accelerated the growth of the plants and encouraged blooming. The % of N, particularly protein-N, in the plants inoculated with active nodule bacteria was much higher than in those plants absorbing only mineral N. A positive N balance was obtained only when biologically fixed N was used as the source of N. Addition of a complete norm of mineral N to the sand resulted in an appreciable loss of N. Active nodule bacteria meet the N requirements of lupins completely under normal symbiotic conditions. A. S. LEVESLEY.

Control of nitrification of ammoniacal nitrogen in soil. L. Soubies, R. Gadet and M. Lenain (*C. R. Acad. Agric. Fr.*, 1962, **48**, 798—803).—The retarding effect of cyanoguanidine (**I**) on nitrification, observed in laboratory experiments, is proportional to the amount of **I** added to the soil (5·4—21 p.p.m.) and is very much greater in soil at pH 8·2 or at summer temp. than in soil at pH 5·0 or at winter temp. **I** does not affect the mineralisation of org. N or of urea; it causes a partial reversion of NO_3^--N to NH_4^+-N. In pot experiments losses of N by leaching were much reduced by applying **I** (11·5% of the applied NH_4^+-N); in field experiments with wheat the efficacy of urea in improving yields was appreciably increased by simultaneous additions of **I**. P. S. ARUP.

Species differences in molybdenum and vanadium requirements and combined nitrogen utilisation by *Azotobacteriaceae*. J. H. Becking (*Plant & Soil*, 1962, **16**, 171—201).—Three of 10 *Azotobacter chroöcoccum* strains tested were unable to utilise V as a substitute for Mo in N_2 fixation. All but one of 20 *A. vinelandii* strains were able to use V, whilst all but one of 20 *A. agile* strains were not able to use V instead of Mo for N_2 fixation. The min. concn. of Mo required for half-max. N_2 fixation decreased in the order *A. chroöcoccum*, *A. agile* and *A. vinelandii*. The *Beijerinckia* species requirement for Mo was variable. In both species Mo was required for N_2

fixation as well as for NO_3^- assimilation. Mo requirements for NO_3^- assimilation in both were about 10% of those for N_2 fixation. Mo was not required for NH_4^+ utilisation. *A. chroöcoccum* strains used NO_3^- in preference to mol. N_2 when both N sources were available. Some *A. vinelandii* strains were able to fix N_2 in the presence of NO_3^- in the medium. *A. agile* strains were unable to utilise NO_3^-. In most *Beijerinckia* strains NO_3^- was not used or was poorly used. A. H. CORNFIELD.

Effect of freezing of soil on denitrification. J. W. McGarity (*Nature, Lond.*, 1962, **196,** 1342—1343).—The efficiency of various freezing treatments on the preservation of denitrifying activity in soil was examined. All the freezing treatments investigated and also air drying increased the denitrifying activity; the lower the temp. of storage, the greater was the denitrifying activity on subsequent incubation. S. A. BROOKS.

Aerobic cellulose bacteria of northern soils. R. A. Zhukova (*Mikrobiologiya*, 1962, **31,** 1054—1060).—Aerobic cellulose bacteria occurring in the soils of the Kola peninsula, largely myxobacteria and vibrions, are examined. The pH range of growth of cellulose bacteria is rather wide, viz. for *Sorangium cellulosum* 3·3—9·2, for *Sporocytophaga myxococcides* 4·2—9·2, and for *Vibrio vulgaris* 5·4—9·2. The optimum pH of cellulose decomposition by *Sporo. myxococcides* was 7·3—8·1, by *Sor. cellulosum* 7·9—8·6, and by *V. vulgaris* 8·2—8·9. Acidity of the medium inhibits the capacity of the bacteria to decompose cellulose. Details of characteristics of the bacteria are given. (16 references.) A. S. LEVESLEY.

Inhibition of nitrification by thiourea and 2-chloro-6-(trichloromethyl)pyridine. D. K. McBeath (*Dissert. Abstr.*, 1962, **23,** 1471).—These two inhibitors were investigated by the soil perfusion technique to evaluate their effectiveness in controlling nitrification and to determine their effect on the nitrifying organisms. Incubation studies in several soils showed that both compounds effectively inhibited nitrification in all soils in which active nitrification can proceed. Neither compound appeared to be very effective in certain soils with pH values below 5·7 because very little nitrification normally occurs in these soils. F. C. SUTTON.

Influence of β-amulose tri-iodide on soil fertility. G. Samuels and F. González-Vélez (*J. Agric. Puerto Rico*, 1962, **46,** 175—182).—In only one of eight trials did β-amulose tri-iodide (' Fertidyne ', claimed to increase soil bacterial activity and thus to increase available nutrients to plants) applied at 2—3 lb./acre with the fertiliser increase the yields of sugar per acre. Fertidyne was also ineffective in increasing sugar yields when applied without fertiliser. Yields of pineapple and yields and quality of tobacco were not affected by application of the material. A. H. CORNFIELD.

Soils and fertilisers. G. W. Cooke (*J. R. agric. Soc.*, 1962, **123,** 134—156).—A review, covering recent work on the status of K in soils, the residual values of fertilisers, analysis of soil and its interpretation, irrigation, use of liquid manures, N, P and K fertilisers and the manuring of crops. (74 references.) A. G. POLLARD.

Mineral elements in plant nutrition and consumption of fertilisers. I. S. Bokde and E. Warren (*Fertil. News*, 1962, **7,** No. 12, 7—15).—A review of the use and development of mineral fertilisers from earliest times up to the present day with details of the increasing consumption in India. Tables and a graph show regional, world and Indian consumption of N, P and K. (17 references.) C. A. P.

Physiological assessment of the nutrient status of plants. I. Preliminary experiments with phosphorus. D. Bouma and E. J. Dowling (*Aust. J. agric. Res.*, 1962, **13,** 791—800).—Leaf area changes in subterranean clover plants, after transfer to nutrient solutions with and without P, give a clear indication, within a few days, of the P status of the soil in which the plants were grown. In field experiments a P-deficient pasture indicated by this technique doubled its dry-matter yield after it had received 3 cwt. of superphosphate per acre. W. ELSTOW.

Effects of fertilisers on the yield and chemical composition of crops grown on organic soils. L. N. Shepherd (*Dissert. Abstr.*, 1962, **23,** 1152).—Studies were made on org. soils to determine (*a*) crop response to variable rates of NaCl and K; (*b*) methods of control of Mg deficiency associated with certain Pascal celery varieties; (*c*) the need for and methods of application of Mo for crop production; and (*d*) the effect of placement of P and Mn fertiliser on the availability of manganese. The field results are recorded. Controlling the symptoms of Mg deficiency on the leaves of celery did not affect, significantly, the yield of the crop. Band application of P gave increased yields, these being the result of enhanced availability of Mn rather than PO_4^{3-} uptake by the crop. F. C. SUTTON.

Balance sheet and residual effects of fertiliser nitrogen in a 6-year study with ^{15}N. S. L. Jansson (*Soil Sci.*, 1963, **95,** 31—37).—Oats were grown in pots in a subsoil poor in total N and of pH 5·8, N being supplied as NH_4^+-N or NO_3^--N, which in the first year was tagged with ^{15}N. In the first-year crop the NO_3^- treatments resulted in higher gross harvested N than did NH_4^+. More ^{15}N was found in NO_3^--treated than in NH_4^+-treated soil. Subsequently uptakes of both forms of N decreased considerably and for the last 4 years were relatively constant with NO_3^- treatments giving slightly the higher values. Uptake of ^{15}N was also relatively constant. The first-year crop contained 35—57% of the ^{15}N added; in the second year this had fallen to 1—8% while in the last 4 years recovery was annually 0·8—1·2% of that added initially. Six years after addition considerable amounts of ^{15}N still remained in the soil. Under constant conditions the half life of the residual fertiliser N may approximate to 20 years. T. G. MORRIS.

Urea–formaldehyde products as manures for agricultural crops. S. N. Datta, M. A. Idnani, V. Iswaran and A. K. Rishi (*Indian J. appl. Chem.*, 1962, **25,** No. 1, 42—46).—The fertiliser efficiencies of 6 urea–formaldehyde (U/F) products, having U/F mole ratios of 1·12—6·75, were compared with that of uncombined urea. Solubility and nitrifiability of the compounds decreased with the decrease of the U/F mole ratio. In pot experiments with paddy and wheat the U/F compounds were not superior to uncombined urea. However, leaching and nitrification experiments showed a product with U/F mole ratio 1·68, to be suitable for use on soils where leaching losses due to rain or irrigation are high. Two compounds of lower U/F mole ratios (1·32 and 1·12) had fertiliser efficiencies too low for economic use. The rate of supply of N to plants may be regulated for a 3-month period, but subsequently the residual N being resistant to decomposition has little residual effect after growing a normal short-duration crop. M. O'LEARY.

Effect of organic matter and moisture levels on the uptake and utilisation of soil- and fertiliser-phosphorus by wheat. N. P. Datta and N. N. Goswami (*J. Indian Soc. Soil Sci.*, 1962, **10,** 263—276).—Wheat was grown in pot culture with four different types of soil which had received various applications of farmyard manure and were maintained at a range of moisture levels. The total and % uptake of soil P increased and those of fertiliser P diminished with increase in org. matter in the soils. The % and total uptakes of both soil- and fertiliser-P increased with rise in soil moisture content. Significant interactions were established between org. matter and moisture contents of soil in relation to total uptake, % fertiliser P in the plant and % utilisation of applied fertiliser P. A. G. POLLARD.

Potassium polymetaphosphate as a fertiliser. H. Munk (*Z. PflErnähr. Düng.*, 1963, **101,** 34—42).—Potassium polymetaphosphate (**I**) is more available in acid than in alkaline soils. In the glassy form **I** passes into solution more readily than when it is cryst. **I** has a residual value similar to that of the orthophosphates. M. LONG.

Effect of continuous application of manures and fertilisers on some physical properties of Punjab soils. J. S. Kanwar and S. S. Prihar (*J. Indian Soc. Soil Sci.*, 1962, **10,** 243—247).—The aggregation, water-holding capacity, hydraulic conductivity and water infiltration rates of these soils were not affected to any greater extent by continuous application of farmyard manure than by that of fertilisers (notably NH_4 phosphates). A. G. POLLARD.

Performance of arable crops under various manurial treatments on blanket peat. E. Grennan and J. Mulqueen (*Irish J. agric. Res.*, 1962, **1,** 251—266).—Nutrient deficiencies for crops on blanket peat are examined. For all crops, N, P, K and Ca are needed. Addition of Cu was essential for cereals, B for sugar-beet, swedes and kale and Mo for brassicas. Results of field trials are presented. A. G. POLLARD.

Fertilisers. Imperial Chemical Industries Ltd. (Inventors: W. C. D'Leny and D. A. Clur) (B.P. 888, 668, 15.6.59).—A particulate or granular fertiliser is rendered non-caking and free flowing by admixture with $\geqslant 10\%$ of K metaphosphate (of particle size <200, preferably $<100\mu$). F. R. BASFORD.

Phosphate fertiliser composition. Hokkaido Tanko Kisen K. K. (B.P. 886,951, 4.3.60. Jap., 6., 19. and 27.3.59).—There is claimed a granulated fertiliser composition comprising a nitrohumic acid (or its alkali metal or NH_4 salt) and a phosphate (preferably a phosphate insol. in water or citric acid). F. R. BASFORD.

Plant Physiology, Nutrition and Biochemistry

Path of carbon in photosynthesis. M. Calvin (*Science*, 1962, **135,** 879—889).—A review. T. G. MORRIS.

Chlorophyll turnover in monocotyledons and dicotyledons. H. J. Perkins and D. W. A. Roberts (*Canad. J. Bot.*, 1963, **41,** 221—226).—Na acetate-1-^{14}C or succinic acid-2,3-^{14}C was fed to mature and immature leaves of four monocotyledons (lily, oats, philodendron and tradescantia), three dicotyledons (red clover, petunia, geranium), a gymnosperm (spruce) and a pteridophyte (Boston fern). Chlorophyll synthesis in the mature monocotyledonous leaves was very slow or non-existent. Considerable amounts of ^{14}C were incorporated into the dehydroporphyrins isolated from the mature leaves of the dicotyledons, the gymnosperm and the pteridophyte. (15 references.) E. M. J.

Upper limit of crop yield. J. Bonner (*Science*, 1962, **137,** 11—15).—The efficiency of the photosynthetic process in leaves is discussed and the overall efficiency is regarded as $\not> 5\%$. The problem of plants in the field with an array of leaves and chloroplasts in the leaves each shading the other is also considered. In areas of high-level agricultural practice the upper limit of possible crop yield under present conditions of CO_2 supply and light is being reached. Plants might be bred for increased conductivity of CO_2 or with different chloroplasts of an improved efficiency. T. G. MORRIS.

Effect of light intensity on the rate of apparent photosynthesis in coffee leaves. M. A. Tió (*J. Agric. Puerto Rico*, 1962, **46,** 159—166).—Max. apparent photosynthetic activity of coffee leaves increased with light intensity up to 2000 ft.-candles, remained at this level up to 6000 ft.-candles and then decreased with further increasing light intensity. The max. rate of photosynthesis was 6138 μg. of CO_2 per h. per sq. dm. of leaf tissue. A. H. CORNFIELD.

Radiation on plant environment and photosynthesis. P. E. Waggoner, D. N. Moss and J. D. Hesketh (*Agron. J.*, 1963, **55,** 36—39).—Laboratory experiments in the past have indicated that plants do not respond to light beyond about 25% of full sunlight. In field tests maize and sunflower showed increasing photosynthesis up to max. sunlight, whilst tobacco and dogweed showed little increase above about 25% max. sunlight. A. H. CORNFIELD.

Rôle of infra-red radiation on the life of plants. I. A. Shul'gin (*Dokl. Akad. Nauk SSSR*, 1962, **146,** 484—487).—A white-tipped variety of radish was grown in daylight supplemented by i.r. radiation from four 500-W lamps 1·5 m. above the plants for 10 h. per day. The radiation reduced the amount of org. material in the edible root although a marked increase in the rate of plant growth was observed. (22 references.) R. A. KEEN.

Water stress and plant growth. P. J. Kramer (*Agron. J.*, 1963, **55,** 31—35).—The significance of water stress within the plant in controlling plant growth is discussed. Methods of measuring the water stress are reviewed. A. H. CORNFIELD.

Transpiration rate reduction in plants with atrazine. D. Smith and K. P. Buchholtz (*Science*, 1962, **136,** 263—264).—Maize and soya-beans grown under controlled conditions were treated with atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) either in irrigation water or as a foliar spray (14—20,000 p.p.m.). Maize (tolerant) showed a max. reduction of transpiration of 44% of the normal rate, while soya-beans (susceptible) showed 67%. Max. reductions usually occurred in 4—6 h., and were due to the closing of leaf stomata. The mode of action of atrazine is discussed. T. G. MORRIS.

Octa–hexa-decanol as a transpiration suppressant. D. B. Peters and W. J. Roberts (*Agron. J.*, 1963, **55,** 79).—Disked in, banded, and broadcast application of mixed fatty alcohols (2 parts octadecanol : 1 part hexadecanol) at 25—500 lb./acre had no effect on yields of and water use by maize. The treatments reduced plant height slightly. A. H. CORNFIELD.

Uptake of dinitrophenol and its effect on transpiration and calcium accumulation in barley seedlings. D. A. Barber and H. V. Koontz (*Plant Physiol.*, 1963, **38,** 60—65).—Ca, DNP and O_2 levels, root viability and transpiration rates were varied for seeds of *Hordeum vulgare* L. var. Arivat. DNP reduced transpiration and initially decreased Ca accumulation but later the amount of Ca accumulated in the shoots was increased. An energy-requiring step appears to be involved in the transport of Ca from the external solution to the xylem vessels of the root. E. G. BRICKELL.

Factors controlling embryo growth and development in barley (*Hordeum vulgare* L.). L. J. La Croix, J. Naylor and E. N. Larter (*Canad. J. Bot.*, 1962, **40,** 1515—1523).—Normal growth and development of barley proembryos occurred in excised intact florets, but not in ovaries from which lemmas and paleas were removed. The lemmas and paleas of the floret apparently contained a substance (referred to as ' hull factor ') which inhibited cell extension and stimulated cell division in the embryo. Evidence was obtained that this stimulation was not due to the provision of a simple energy source, e.g., sucrose. In absence of the ' hull factor ', ovaries cultured *in vitro* contained embryos having nuclei in which the DNA content was equivalent to the tetraploid level, while mitosis was almost completely lacking. A similar embryo growth-stimulation was obtained when a single leaf was left on an excised barley spike from which lemmas and paleas were removed. J. L. WALPOLE.

Vitamins in germination. Distribution of inositol during germination of dwarf bean, *Phaseolus vulgaris*. L. N. Gibbins and F. W. Norris (*Biochem. J.*, 1963, **86,** 64—67).—The amounts of inositol in each anatomical part of the bean seedling are determined, by microbiological methods, at each of six stages during early development of the plant. The total inositol in the seedling decreases by 40% during the first 2 weeks of growth in the dark. Inositol is liberated from phytin in the cotyledons and is translocated to the growing parts of the plant. During early development of the plant the inositol content of the plumules increases 20-fold ; most of this increase is due to free inositol. About an 8-fold increase occurs in the roots but this is due mainly to bound forms. There is practically no alteration in the amount of inositol in the testas during germination. The content of total inositol is increased in the hypocotyls and epicotyls ; this is due mainly to an influx of free inositol. (17 references.) J. N. ASHLEY.

Course of cation absorption by plant tissues. E. Epstein, D. W. Rains and W. E. Schmid (*Science*, 1962, **136,** 1051—1052).—Absorption of ^{86}Rb by excised barley roots from solution was a strictly linear function with time ; it was depressed by decrease in temp. There was no evidence that, when Ca was present, there was any appreciable period of equilibration before the onset of steady state metabolic absorption of Rb. The Rb absorbed reached a concn. in excess of that in the external solution. T. G. MORRIS.

Morphogenetic influence of (CO_2 + HCO_3^-) on roots. G. Geisler (*Plant Physiol.*, 1963, **38,** 77—80).—Pea roots grown in water culture were treated with varying levels of CO_2 ; concn. within the range 30—150 mg./l. accelerated root development considerably. Dry-matter production in roots was significantly favoured by moderate CO_2 levels. E. G. BRICKELL.

Anion exchange in roots. A. V. Peterburgskiĭ and G. L. Nelubova (*J. Sci. Fd Agric.*, 1963, **14,** 186—187).—A modification of Piper's method for the determination of anion-exchange capacity (**I**) of soils with roots of seedlings is described. The phosphate adsorbed from NH_4 phosphate solution is extracted with NaOH and determined colorimetrically. The error of analysis for four replicates was usually 1—3 but occasionally up to 7%. **I** in roots of oats and barley drops during growth of seedlings and, at the same age, roots of peas, sunflower and maize give higher values than those of oats and barley. E. M. J.

Mechanism of anion uptake by plant roots. V. Chloride uptake by maize root tips. M. M. Elgabaly and R. Handley (*Plant & Soil*, 1962, **16,** 165—170).—The uptake of Cl^- by the non-vacuolated tissue of the zone of cell division of the root tip of maize from Cl^- adsorbed on resin, $CaCl_2$ and NaCl and from their equilibrium filtrates was studied. There was greater uptake of Cl^- from the resin–NaCl system than from the NaCl filtrate, but little difference in Cl^- uptake between the resin–$CaCl_2$ system and the $CaCl_2$ filtrate. Pre-treating the root segments with a Na-chelating agent prior to immersion in the absorption solutions caused a reduction in Cl^- uptake relative to that of the untreated material. The reduction was more pronounced from NaCl than from $CaCl_2$. A. H. CORNFIELD.

Kinetic studies of anion absorption by potato slices at 0°. I. R. MacDonald and G. G. Laties (*Plant Physiol.*, 1963, **38,** 38—44).—The time course of Cl^-, Br^- and PO_4^{3-} uptake at 0° follows a two-component absorption pattern—an initial period of rapid uptake (the absorption shoulder) followed by a period of steady-state uptake. The absorption shoulder is specific for a given ion or ionic species and may reflect the filling of absorption sites in the cytoplasm while the steady state is indicative of vacuolar penetration. E. G. BRICKELL.

Genetic variation in root cation-exchange capacity of ryegrass. M. C. H. Mouat (*Plant & Soil*, 1962, 16, 263—265).—The cation-exchange capacity (C.E.C.) of the roots of seven clones of ryegrass, grown from tillers for 5 weeks in sand culture, ranged from 17·2 to 24·9 mequiv. per 100 g., and there were significant clonal differences in C.E.C. These differences are probably sufficiently great to affect the balance of nutrients absorbed by the plants. A. H. CORNFIELD.

An artifact in plant autoradiography. E. Levi (*Science*, 1962, **137,** 343—344).—When primary leaves of bean plants which had been grown in complete Hoagland solution under constant conditions of light, temp. and humidity and treated with radioactive P were dried at 80° in an oven, the veins appeared to be depleted of tracer,

pptn. of which appeared to have occurred in interveinal areas. P was apparently mobile in killed tissues. When, however, the leaf was freeze-dried there was an even distribution of the isotope in the leaf. When leaves were placed in an oven between plates which had holes in them an accumulation of P was found near the holes where drying had been faster. T. G. MORRIS.

Potassium–magnesium antagonism in soils and crops. E. Welte and W. Werner (*J. Sci. Fd Agric.*, 1963, **14**, 180—186).—The effects of increasing the concn. of H^+, K^+, NH_4^+ and Ca^{2+} ions in the substrate on the Mg-uptake by the plant are discussed. Depressive effects of the different cations are additive, e.g., the K/Mg antagonism is the more pronounced the lower is the pH. An inadequate supply of Mg in soil adversely affects yields. E. M. J.

Effects of chelates on the concentration of magnesium in apple trees. W. L. Koukkari (*Dissert. Abstr.*, 1962, **23**, 1477).—Orchard and greenhouse experiments were employed to study the effcts of the chelating agent, the Na_3 salt of *N*-hydroxyethylethylenediaminetriacetic acid and its iron chelate, on the concn. of Mg in young apple trees. Mg did not appear to move readily from the basal leaves. F. C. SUTTON.

Iron uptake–transport of soya-beans as influenced by other cations. J. S. Lingle, L. O. Tiffin and J. C. Brown (*Plant Physiol.*, 1963, **38**, 71—76).—With increasing concn. of the interfering cations (Mn, Cu, Ca, Mg and K + Rb) in the nutrient Fe concn. in the stem exudate first increased and then decreased to very low levels. Small concn. of Zn however depressed the absorption of Fe over the entire range tested. Zn was the strongest interfering ion studied in decapitated plants; it also interfered with the uptake–transport of Fe by intact plants. E. G. BRICKELL.

Analysis of iron chelates in plant extracts. II. Ferric ethylenediaminebis-(o-hydroxyphenylacetic acid). D. G. Hill-Cottingham and C. P. Lloyd-Jones (*J. Sci. Fd Agric.*, 1963, **14**, 171—175; cf. J.S.F.A. Abstr., 1961, i, 256).—The behaviour of ethylenediaminebis-(*o*-hydroxyphenylacetic-acid) (EDHPA, Chel 138) and its Fe^{III} chelate (Fe-EDHPA) with activated C and a cation-exchange resin is described. Fe-EDHPA in aq. solution is adsorbed by activated C and eluted by ethanolic NH_3. EDHPA in neutral or acid solution is adsorbed on the H^+ form of the cation-exchange resin Zeocarb-225 and is eluted completely by ethanolic NH_3. When Fe-EDHPA solution is acidified with HCl to pH 1 all the EDHPA is retained by the resin. Fe-EDHPA can be identified by paper chromatography. A modification of the previously-proposed procedure for the detection of Fe-EDTA in plant extracts is also described. E. M. J.

Long-term field experiments with small applications of boron. M. Ødelien (*Soil Sci.*, 1963, **95**, 60—62).—Annual applications of B (0—200 g./acre) to a soil of marine origin with average annual temp. of 5·5° and rainfall of 298—1007 mm. were made over 23 years. Various crops were grown at intervals but swedes were grown every 5 years and examined for signs of B-deficiency. On control plots B-deficiency increased markedly over the test period, but 50 g. of B per acre was sufficient to prevent this. Amounts >50 g., e.g., 400 g./acre, were injurious especially on unlimed soil. T. G. MORRIS.

Uptake of rubidium by plants from cation-exchange resin suspensions and their equilibrium solutions. C. H. E. Werkhoven (*Dissert. Abstr.*, 1962, **23**, 388).—A laboratory study was undertaken to evaluate Rb absorption by plants from suspensions of a cation-exchange resin and their equilibrium solutions as applied at small distances, defined by high passivity filters, separating the roots from the exchanger. The existence of two-phase effects on ion absorption by plants is indicated and the mechanism of the process is discussed. F. C. SUTTON.

Effects of iodide and iodate on plant growth. G. W. F. H. Borst Pauwels (*Plant & Soil*, 1962, **16**, 284—292).—Growth of oats was depressed in water culture solutions containing >0·75 p.p.m. of I as I^- or >2·25 p.p.m. as IO_3^-. The rate of uptake of I from I^- was more than double that from IO_3^-. The I^- and IO_3^- forms had similar depressing effects on growth of oat roots. A. H. CORNFIELD.

Plant uptake of sulphur. J. Jensen (*Soil Sci.*, 1963, **95**, 63—68).—A discussion with particular reference to soils of Denmark. Pot tests are reported in which a variety of plants were grown in sand outside and in a well-ventilated greenhouse (i.e., no rain but free access of air) supplied with nutrients and two levels of S tagged with ^{35}S. In general the more the S added as fertiliser the less was the S absorbed from the air or rain. T. G. MORRIS.

Plant silica, an abrasive constituent of plant matter? R. B. Sharp (*J. agric. Engng Res.*, 1962, **7**, 214—220).—The silica particles present in plant material were extracted in what was considered unchanged form by dissolving the plant material with dil. aq. CrO_3. The extracted particles were predominantly opaline silica, SiO_2,nH_2O. The proportion and hardness of opal in the plant species studied and damage to the opaline silica structures during milling are consistent with the abrasive properties of plant matter. A. H. CORNFIELD.

Beryllium and the growth of bush beans. E. M. Romney, J. D. Childress and G. V. Alexander (*Science*, 1962, **135**, 786—787).—Bush bean plants were grown in culture solution adequately supplied with major and minor nutrients, to which Be, up to 5 p.p.m., was added. The dry wt. of the plants decreased with increasing Be levels. With Be, at 3 and 5 p.p.m. the roots became brown and stunted; foliage was also stunted but the colour remained normal; flowering was earlier and mature pods were fewer. Be accumulated in the root tissues. Ca levels in all parts of the plant decreased with increasing Be levels. Mg levels were lowered in roots and stems but were unaffected in leaves and fruits. P concn. tended to increase with increasing Be. T. G. MORRIS.

Plant nutrition and yield curves. F. Steenbjerg and S. T. Jakobsen (*Soil Sci.*, 1963, **95**, 69—88).—A review and discussion. T. G. MORRIS.

Association of plant vigour with physical stature and chemical content of desert plants. C. W. Cook and C. J. Goebel (*Ecology*, 1962, **43**, 543—546).—The chemical composition and size of individual plant organs of species growing under conditions leading to high or low vigour are recorded. In general high vigour was associated with higher contents of ether extractives, lignin and gross energy and low vigour with higher contents of protein, ash, Ca and P. A. G. POLLARD.

Fractionation of nitrogenous compounds from maize leaves. J. G. Dickson and J. L. Lords (*Nature, Lond.*, 1962, **196**, 1099—1100).—Chromatography on columns of diethylaminoethyl cellulose of purified protein extracts obtained from maize leaves with ice cold 0·05M-phosphate buffer pH 6·8 gave a much better resolution than did electrophoresis. Gradient elution was used with 0·005M-phosphate as the stating solution and 0·5—2M-NaCl solution as the limiting solution. Differences in the protein elution patterns of two inbred lines of maize were detectable as well as in the same inbred line growing at 20 and at 28°. W. ELSTOW.

Variation in the content of phosphorus compounds in plants grown under anaerobic conditions. G. M. Grineva (*Dokl. Akad. Nauk SSSR*, 1962, **146**, 475—477).—Maize and sunflower plants, at the 2—3 leaf stage of growth, were subjected to a 6-h. treatment in a dark box in which N_2 was substituted for air. The plants were then treated with liquid N_2, dried and analysed for P. Org. P (extracted by 5% trichloroacetic acid) in the roots and leaves was reduced by up to one third (except in the leaves of the maize plants) by the exposure to an O_2-free atm. Inorg. P was not significantly affected. Retardation of the water absorption process by plants grown in anaerobic conditions is connected with the suppression of the oxidation of P compounds. (16 references.) R. A. KEEN.

Determination of certain phosphate compounds in plant extracts. J. Barker, F. A. Isherwood, R. Jakes, T. Solomos and M. E. Younis (*Nature, Lond.*, 1962, **196**, 1115).—The successful determination of glucose- and fructose-6-phosphate, fructose diphosphate, dihydroxyacetone phosphate, 3-phosphoglycerate and phosphoenolpyruvate in extracts of potatoes, strawberry leaves, bananas, tomatoes and apples by enzymic methods is reported. In bananas a correlation was observed between changes in the rate of respiration during ripening and the fructose diphosphate content. (10 references.) W. ELSTOW.

Effect of toxins of soil fungi on nitrogen and amino-acid contents of plants. T. G. Mirchink, F. G. Kopÿsskaya and K. P. Greshnÿkh (*Mikrobiologiya*, 1962, **31**, 669—676).—Intake by pea, vetch and wheat plants of toxic substances formed by saprophytic soil fungi of genus *Penicillium* was studied. Effect of toxins on total N and distribution of N in plants was observed. Toxins, extraction of which is described, were from *P. cyclopium*, West (**I**), raw material of relative activity 640 units/ml, *P. martensii*, Biourge (**II**) and *P. purpurogenum*, Stoll (**III**). **II** and **III** were cultural liquids with activity 32 units/ml. Doses, insufficient to kill plants, applied as solutions or through soil, rapidly penetrated into above-ground parts of plants and did not remain in roots. Effects of each toxin on total N, N in 13 free amino-acids and in protein were determined in the plants. **III** was most powerful in reducing N content of all plants. Toxins **I** and **II** act powerfully on N compounds of pea and vetch but have little effect on those of wheat. (12 references.) P. W. B. HARRISON.

B-vitamins in root exudates of cotton. C. B. Sulochana (*Plant & Soil*, 1962, **16**, 327—334).—*Fusarium* wilt-sick and wilt-free soils both contained choline, pyridoxine, *p*-aminobenzoic acid, biotin and inositol, and the wilt-free soil also contained thiamine. Thiamine, biotin, pyridoxine and *p*-aminobenzoic acid were found in higher amounts in the root exudates of diploid than in those of amphidiploid cotton strains. A. H. CORNFIELD.

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Isolation and identification of a seleno-amino-acid from maize. A. L. Jacobs (*Dissert. Abstr.*, 1962, **23,** 1504).—Maize plants were grown for one month in a culture solution containing K selenate. An aq. extract of the dried harvested plants was subjected to separation by ion-exchange resin and paper column chromatography. A neutral amino-acid was separated and identified as the Se analogue of cystine. A semi-micro technique for the quant. determination of Se was devised which required only small amounts of plant material. The Se was separated from interfering elements by distillation and was determined by reaction with an excess of Na thiosulphate followed by titration with a standard I solution. F. C. SUTTON.

Further improvements in stationary platinum electrode of Haxo and Blinks. J. Myers and J.-R. Graham (*Plant Physiol.*, 1963, **38,** 1—5).—Two further modifications, the rapid flow of external solution and the use of massive Pt anodes, which increase stability and precision of measurement, are described. E. G. BRICKELL.

Design of a rotary shaking machine. L. Machlis (*Plant Physiol.*, 1963, **38,** 35—37).—A tier of shelves, each shelf being attached to a central tube by set screws, is given rotary agitation by a shaft running through the central tube on eccentric bearings. A stabiliser at the top prevents the supporting column and the shelves from complete rotation. E. G. BRICKELL.

Conversion of indol-3-yl-acetaldoxime into -acetonitrile by plants. S. Mahadevan (*Arch. Biochem. Biophys.*, 1963, **100,** 557—558).—The immediate precursor in the biogenesis of indolylacetonitrile (**I**) is considered. Indolylacetaldoxime (**II**) can be converted into **I** and indolylacetic acid (**III**) by several members of the Moniliales. The reaction is enzymic, **I** extracted from the tissues giving the typical blue-violet colour with Salkowski's reagent. Barley and cabbage leaf tissue also converted **II** to **III** but **I** could not be demonstrated by chromatography. Possibly the conversion of **I** to **III** is faster than the rate of **I** formation and hence **I** does not accumulate. C. V.

Inactivity of hexitols and hexoses in biosynthesis of auxin from tryptophan. S. A. Gordon and E. Buess (*Plant Physiol.*, 1963, **38,** 11).—D-Sorbitol, *meso*-inositol, D-glucose, fructose and sucrose were tested at concn of 10^{-2}M in a suitable incubation medium. No auxin activity was observed during incubation with tryptophan in the absence of enzyme; probably a carbocyclic as well as an unsaturated ring structure is required for formation of the requisite oxidant. E. G. BRICKELL.

Independence of effects of auxin on cell wall methylation and elongation. R. Cleland (*Plant Physiol.*, 1963, **38,** 12—18).—In Avena coleoptile, 0·05M-ethionine causes some inhibition of elongation but does not eliminate the effect of auxin on elongation. This auxin-induced elongation can occur under conditions where auxin-induced methylation of pectic substances is completely suppressed. In the absence of added sugars, auxin does not cause any measurable increase in amount of any of the pectic fractions. E. G. BRICKELL.

Growth-modifying and antimetabolic effects of amino-acids on chrysanthemum. S. S. Woltz (*Plant Physiol.*, 1963, **38,** 93—99).—Four of 20 natural amino-acids (L-isoleucine, L-leucine, L-methionine and L-valine) applied to the root zones of chrysanthemum plant had significant growth modifying effects which are described. Seven synthetic amino-acids (DL-alloisoleucine, DL-norleucine, DL-ethionine, DL-methionine, DL-valine, DL-isovaline and DL-norvaline) had similar effects. E. G. BRICKELL.

Plant growth-regulating substances. XVII. S-Esters of dithiocarbamates derived from amino-acids. K. Rothwell and R. L. Wain (*Ann. appl. Biol.*, 1963, **51,** 161—167).—A range of *N*-substituted *S*-esters of dithiocarbamates derived from amino-acids was synthesised and their activities were assessed in the wheat cylinder, pea segment, and pea curvature tests. The highest activity in all tests was shown by *S*-methyl-*N*-methyl-*N*-carboxymethyldithiocarbamate. *N*-Substituents other than CH_3 reduced the activity, whereas a group other than CH_3 attached to the S destroyed activity. Activity is discussed in relation to the nature of the N—C bonding within the molecules. A. H. CORNFIELD.

Peach mesocarp explant enlargement and callus production *in vitro*. N. F. Sommer, M. V. Bradley and M. T. Creasy (*Science*, 1962, **136,** 264—265).—Effects of various growth-regulating substances on the explants in nutrient media are examined. Abundant callus production occurred only with kinetin or coconut milk. In presence of kinetin, naphthylacetic acid (**I**) hastened callus formation, but callus developed in some cases in absence of **I**. T. G. MORRIS.

Effect of rotation on flowering response of *Xanthium pennsylvanicum*. T. Hoskizaki and K. C. Hamner (*Science*, 1962, **137,** 535—546).—*Xanthium* plants were turned at 0·25 r.p.m. about a horizontal axis at a distance of 10—20 in. from the axis. The average light intensity at the plant surface was 800 ft.-candles with a variation between 350 and 1000. Temp. was maintained at 24—27°. During rotation the plants were subjected to 2 long days and 3 days of variable periods of light and dark, and then 1 long day. All plants subjected to a 9·75-h. dark period and then rotated were vegetative, while the corresponding stationary controls had an average flowering stage of 5. Even with 10·25 h. of darkness the flowering of the rotated plants was much reduced. Floral initiation appears to be most sensitive to rotation prior to the inductive dark period. T. G. MORRIS.

Chemical regulation of flower sex expression and vegetative growth in *Cucumis sativus*, L. W. D. Mitchell and S. H. Wittwer (*Science*, 1962, **136,** 880—881).—Germinated seedlings of monoecious and gynoecious cucumber were transferred to solution cultures immediately following cotyledon expansion. Allyltrimethylammonium bromide (AMAB) and gibberellin A_3 (GA_3) were added to the cultures in varied mol. concn. and plants were grown at 21—23° with 9—11 h. lighting. The two chemicals induced opposite responses in flower sex expression, tendril formation and vegetative extension. In the control monoecious plants, staminate flowers were produced exclusively on the first 17 nodes. Similar plants treated with 5×10^{-4}M-AMAB formed pistillate flowers at 9 of the first 20 nodes with the first at the second node. Some plants produced exclusively pistillate flowers after the ninth node. Tendril formation was delayed until the 11th node. GA_3 at 10^{-3}M caused reversion from pistillate to staminate flowers in gynoecious cucumbers up to the 10th node. With 10^{-4}M-GA_3 staminate flowers were continuous from the second to the fourth nodes with some staminate flowers before the 10th. Some plants produced both types. GA_3 at 10^{-5}M or higher concn. changed the position of the first tendril from the third to the second node. T. G. MORRIS.

Suppression of floral induction by inhibitors of steroid biosynthesis. J. Bonner, E. Heftmann and J. A. D. Zeevaart (*Plant Physiol.*, 1963, **38,** 81—88).—Plants of *Xanthium pennsylvanicum*, Wall, and *Pharbitis nil*, Chois, were treated with the following inhibitors of cholesterol biosynthesis—β-diethylaminoethyl diphenylpropylacetate hydrochloride (**I**), 2,2-diphenyl-1-(β-dimethylaminoethoxy)-pentane hydrochloride (**II**), tris-(2-dimethylaminoethyl) phosphate trihydrochloride (**III**), tris-(2-diethylaminoethyl) phosphate trihydrochloride (**IV**) and 1-[(4-diethylaminoethoxy)phenyl]-1-(*p*-tolyl)-2-(*p*-chlorophenyl)ethanol (**V**). **I** and **II**, which inhibit conversion of mevalonic acid to squalene cause yellowing of treated leaves; only **II** inhibits flowering. **V**, which inhibits the final steps of cholesterol synthesis, does not affect floral induction, but causes severe leaf necrosis. **III** and **IV**, which inhibit conversion of lanosterol to cholesterol, are powerful inhibitors of floral induction for both plants. Flower hormone synthesis is the process inhibited by **IV** and no substance has as yet been found capable of overcoming it. E. G. BRICKELL.

Relationships of gibberellin and auxin to thermal induction of flowering in *Lolium temulentum*, L. M. L. Peterson and L. E. Bendixen (*Crop Sci.*, 1963, **3,** 79—82).—Germinating seeds of *L. temulentum* (darnel) were exposed to low temp. (3°) on filter papers moistened with aq. gibberellin or indolylacetic acid for varying periods before planting in soil. Cold-induction of flowering was measured by reduction in no. of days to heading and no. of leaves and tillers present at heading. Gibberellic acid (**I**) lowered the no. of leaves at heading only after intermediate cold treatments (7 days). IAA in small concn. slightly increased leaf production at heading but higher concn. after cold treatment had the reverse effect which was largely overcome by **I**. In the cold-induction of flowering probably neither **I** nor IAA are major factors. A. G. POLLARD.

Influence of kinetin and gibberellic acid on growth and alkaloid patterns in *Datura meteloides*. D. G. Ambrose and L. A. Scinchetti (*J. pharm. Sci.*, 1962, **51,** 934—938).—Treatment with four weekly doses of gibberellic acid (25 μg.) increased the height and dry wt., and reduced the alkaloid content in plant organs and the content chlorophyll *a* and *b*. Kinetin produced shorter plants, reduced dry wt., increased chlorophyll *a* and *b* content but made no significant change in alkaloid content. (28 references.) B. H. COUPE.

Gibberellin-like substances in plants. J. Kato, W. K. Purves and B. O. Phinney (*Nature, Lond.*, 1962, **196,** 687—688).—Some evidence has been obtained for the occurrence of gibberellin-like substances in gymnosperms and ferns. S. A. BROOKS.

Mode of action of growth-retarding chemicals. S. Kuraishi and R. M. Muir (*Plant Physiol.*, 1963, **38,** 19—24).—Effects of the growth-retarding chemicals 2-chloroethyltrimethylammonium chloride and tributyl-2,4-dichlorobenzylphosphonium chloride in combination with gibberellin on leaf growth of *Raphanus sativus*, L. were tested. Inhibition was greatest with the highest concn. of gibberellin and the same effects were found when IAA was present. Growth retardation appears to be due to the lowering of auxin level in the plant. E. G. BRICKELL.

Retardation of plant growth by a new group of chemicals. J. A. Riddell, H. A. Hageman, C. M. J'Anthony and W. L. Hubbard (*Science*, 1962, **136**, 391).—Foliar sprays of both *N*-dimethylaminomaleamic acid (CO11) and succinamic acid retarded the growth of a wide variety of plants. CO11 in foliar sprays (1000 p.p.m. in water) retarded growth by reducing intermode length of pinto beans by approx. 50%. The rate of development was not affected. Potatoes sprayed 20 days after planting were 48% shorter than were controls at harvest and the tubers after storing for 5 months at 7° sprouted within 7 days; one month later the sprouts from these tubers were only half the height of those from untreated plants, indicating a long residual action. T. G. MORRIS.

Tumour formation in interspecific hybrids of *Lilium*. S. L. Emsweller, S. Ashen and J. Uhring (*Science*, 1962, **136**, 266).—Tumours formed on germinating seeds of *Lilium speciosum* 'Album' (*L. auratum* hybrid) are examined. When sterilised in Ca hypochlorite the seeds grew normally. Water extracts of unsterilised seeds inhibited the growth of wheat coleoptiles. Ferulic acid, present in the water extracts, also inhibited growth of embryos when incorporated in the growth medium (5×10^{-4}M), but when added at the rate of 5×10^{-5}M, caused tumours to form. Seeds of this hybrid contained 11 μg. of ferulic acid per g. wet wt., but a different, easily germinated hybrid contained only 1 μg./g. T. G. MORRIS.

Growth responses of *Avena* stem segments to various sugars. P. B. Kaufman, J. M. Katz and M. E. Yoder (*Nature, Lond.*, 1962, **196**, 1332—1333).—The extension of the internode portion of *Avena sativa* L. stem segments was measured in the presence of various sugars. Min. growth response was found with galactose, mannose, xylose, lactose and arabinose, intermediate response with ribose, maltose, glucose, D-sorbitol, myo-inositol and D-mannitol and max. response with sucrose and fructose. S. A. BROOKS.

Crops and Cropping

Influence of date of seeding and nitrogen rates on winter wheat varieties in eastern Oregon. L. K. Beutler and W. H. Foote (*Agron. J.*, 1963, **55**, 4—6).—Some semi-dwarf winter wheat selections had a much earlier optimum seeding date than had currently grown varieties, as measured by straw and grain yields. This adaptation to earlier autumn seeding dates also influenced differential responses by varieties to rates of N fertiliser. A. H. CORNFIELD.

Effect of seeding rate, row spacing and rate and placement of fertiliser on winter wheat performance in Michigan. K. L. Kinra, H. D. Foth, L. S. Robertson and H. M. Brown (*Agron. J.*, 1963, **55**, 24—37).—Autumn culm counts and autumn vigour ratings of autumn-sown wheat were affected more by varying seeding rates, row spacing, fertiliser rate and fertiliser placement than were yield, lodging score and test wt. High fertiliser rates, particularly with seed contact placement, resulted in reduced yields. The importance of establishing a good stand in the autumn was shown by the positive correlations between autumn culm count and yield in 3 of 4 experiments. A. H. CORNFIELD.

Effect of nitrogenous fertiliser on the quality of Atle wheat. P. A. Spillane (*Irish J. agric. Res.*, 1962, **1**, 237—250).—The increased protein content of Atle wheat resulting from applications of N fertiliser was not accompanied by a parallel change in baking quality. The fertiliser effect on carbohydrate fractions of the grain was greater than that on the protein and tended to influence baking quality adversely, notably in reducing loaf vol. and causing inferior crumb quality. Max. increases in the protein content of the grain resulted from application of N at the stage of ear emergence; the depressive effect on baking quality followed top-dressing with N at the 4- or 5-leaf stage. A. G. POLLARD.

Nitrogen metabolism and water regime of rice plants affected by 'brusone' disease. F. Zsoldos (*Plant & Soil*, 1962, **16**, 269—283).—Diseased tissue was higher in total N and protein-N (dry basis) than was healthy tissue, and protein-N as % of total N was lower in diseased than in healthy tissue. The physiological activity of the root system of brusone-susceptible and -resistant varieties were markedly different, particularly in the later stages of growth. Excessive application of N encourages the development of the disease by increasing the shoot/root ratio and disturbing the water regime and mineral balance. Physiological drought occurs in years when the disease is serious. A. H. CORNFIELD.

Micronutrient deficiency symptoms of rice grown in nutrient culture. A. Q. M. B. Karim and J. Vlamis (*Plant & Soil*, 1962, **16**, 347—360).—Well-defined deficiency symptoms of Mn, Zn, B and Cl were induced in rice in nutrient cultures. Cu and Mo deficiencies appeared late and reduced seed production; the seed produced failed to germinate. A. H. CORNFIELD.

Growth and chemical composition of maize plants as related to nitrogen and potassium nutrition. A. V. Barker (*Dissert. Abstr.*, 1962, **23**, 1474—1475).—With maize plants in gravel culture trials in the greenhouse and in an outdoor hydroponics installation, the uptake and distribution of N in the plants were studied in relation to several varying nutritional regimes. Total amounts of free amino-acids were vastly increased by higher levels of N and NH_4^+ nutrition. Higher K rates reduced the total free amino-acids in the plants at any given N level. F. C. SUTTON.

Irrigation and nitrogen effects on sweet corn row numbers at various growth stages. H. A. Schreiber, C. O. Stanberry and H. Tucker (*Science*, 1962, **135**, 1135—1136).—The no. of kernels per row in ears of *Zea mays* L. in the field was affected by environmental conditions. Row no. were unaffected by irrigation at any stage of growth but they were increased significantly by applications of as little as 25 lb. of N/acre at planting. T. G. MORRIS.

Nitrate reductase activity, protein content and yield of four maize hybrids at varying plant populations. J. F. Zieserl, W. L. Rivenbark and R. H. Hageman (*Crop Sci.*, 1963, **3**, 27—32).—Effects of partial shading (due to closer spacing) of maize plants on the NO_3^--reductase activity (**I**) of the leaves is examined. In upper leaves **I** and protein content exeeed those in the more shaded leaves; **I** and NO_3^- content were inversely related. Leaf-**I** and -protein showed parallel changes with advancing development of the plants. The N metabolism of plants is probably a major factor in the reduced yield per plant resulting from closer spacing and consequent shading. A. G. POLLARD.

Maize as a silage crop in the West Midlands. M. Eddowes (*J. R. agric. Soc.*, 1962, **123**, 55—62).—Varietal differences in % of total dry matter in maize crops present in the ears are recorded. Effects of pre-emergence weed control with atrazine on growth and yield of the crop and of time of sowing and seasonal factors on the feeding value of the silage are examined. A. G. POLLARD.

Maize as a silage crop in south-east England. E. S. Bunting (*J. R. agric. Soc.*, 1962, **123**, 46—54).—Recent work on the effects of time of sowing and of density of plants/unit area on yields of fresh material and dry wt. is discussed. A. G. POLLARD.

Potato growing today. C. V. Dadd (*J. R. agric. Soc.*, 1962, **123**, 33—45).—A review of current practices. Cleaning of seed, manuring, cultivation, weed control, plant protection, harvesting and storage of the crop are considered. A. G. POLLARD.

Effect of light on formation and composition of anthocyanins in potato tubers during vernalisation. S. A. Stanko (*Dokl. Akad. Nauk SSSR*, 1962, **146**, 480—483).—Potato plants were subjected to normal daylight conditions of growth or 12 h. illumination from 9 a.m. or 12 h. illumination from 9 p.m., the remainder of the time being spent in darkness, over periods of 45 days during June—July. The anthocyanins extracted from the tubers by 5% HCl in methanol totalled 119, 113 and 42 mg. per 100 g. dry material respectively. Examination of the plants showed the absence of cyanidin in those illuminated during normal daylight hours. (12 references.) R. A. KEEN.

Effects of different soils on composition and growth of sugar beet. P. J. Goodman (*J. Sci. Fd Agric.*, 1963, **14**, 196—203).—Contrasts in growth, composition and yield on two different soil types with that at Rothamsted (*R*) are discussed. A silty loam had a high nutrient status, including a Na level ≡ 3 cwt. of salt/acre/year while an oolitic limestone soil had limited K and P availability. On the clay with flints (*R*), in 1960, K was a limiting factor, while in 1961 N was limiting. N application limited availability of P to roots. The yield of different centres and years was closely related to N-supply as measured by N-uptake. Losses particularly of Na and K from roots occurring towards the end of the growth period may be important in relation to juice purity. E. M. J.

Sugar beet yields on fallowed and non-fallowed land on two soil types. R. A. Hedlin and K. Schreiber (*Agron. J.*, 1963, **55**, 10—12).—The main effect of fallowing a clay loam and a very fine sandy loam was to increase NO_3^--N by 30—80 lb./acre to the 4 ft. depth. This led to a lower response by sugar-beet to N application on fallowed as compared with non-fallowed soil. A. H. CORNFIELD.

Dry-matter content of meadow grass. W. D. Jagtenberg (*Landbouw-Voorlichting*, 1962, **19**, 626—636).—Numerous results obtained during 1957—59 show variations of 9—36% in the dry-matter content and an average difference of 6·7% as between very wet and dry grass. Data are given for the correction of results obtained for 'very wet', 'wet', or 'damp' grass to a 'wind-dry' grass basis A constant average difference of 1·6—1·7% is found between any two consecutive grades of batches judged by the above scale. The corrected results for numerous cuttings made under various conditions of growth show an inverse relationship (linear or slightly

curvilinear) between the dry-matter content and the rate of growth. In order to maintain milk yields during cool and wet periods in autumn it is recommended to avoid, as far as possible, pasturing on lush grass. P. S. ARUP.

Temperature effect on potassium uptake and respiration of warm- and cool-season grasses and legumes. R. E. Worley, R. E. Blaser and G. W. Thomas (*Crop Sci.*, 1963, **3,** 13—16).—The rate of uptake of K by excised roots of warm-season plants (Sudan grass, soya-bean) from nutrient solution exceeded that for cool-season plants (rye, peas); pea roots lost K at 35°. Endogenous K per g. in legume roots was much higher than in grass roots. At 5° the K uptake of grasses exceeded that of legumes; soya-bean roots lost K at 5—15°. In general respiration of the roots increased with temp. except that at 30° the CO_2 release from soya-bean and rye declined somewhat before rising again at 35°. A. G. POLLARD.

Effects of previous superphosphate applications on the pasture environment and the response by pasture to a current dressing. K. D. McLachlan and B. W. Norman (*Aust. J. agric. Res.*, 1962, **13,** 836—852).—On soils of granitic, sedimentary and basaltic origin subterranean clover pasture production is limited by a P deficiency and not a S deficiency as suggested by previous evidence. There is scope for altering the P and S content of superphosphate to produce a more efficient fertiliser for particular stages in pasture development and for particular soils. (16 references.) W. ELSTOW.

Soil and herbage levels in relation to yield. R. G. Hemingway (*J. Sci. Fd Agric.*, 1963, **14,** 188—195).—K analyses in soil and herbage over a period of 3 years of herbage production under various fertiliser treatments are recorded. A combination of N and K was essential for maintaining yields and 2 cwt. of KCl/acre/annum was not adequate if 12 cwt. of $(NH_4)_2SO_4$/acre were applied each year. Additions of K to depleted plots in the third year greatly improved yields. The mean release of non-exchangeable K over the 3-year period was 2·7 mg./100 g. of soil which was only 22% of the initial exchangeable K value. K concn. in the herbage fell to 0·4% K on plots receiving N only and to 1·1% K where N and K were given. E. M. J.

Yield response surfaces, isoquants and economic fertiliser optima for Coastal Bermuda grass. L. F. Welch, W. E. Adams and J. L. Carmon (*Agron. J.*, 1963, **55,** 63—67).—Yield data from a 4 × 4 × 4 NPK factorial experiment were used to obtain a yield equation by multiple regression. The yield equation was then used to calculate response surfaces, isoquants and economic fertiliser optima. A. H. CORNFIELD.

Effect of indol-3-ylbutyric acid on shoot development and rooting of Bermuda grasses. C. S. Hoveland (*Agron. J.*, 1963, **55,** 49—50).—Treatment of Suwanee Bermuda grass rhizomes with 0·1% indol-3-yl-butyric acid (**I**) dust before planting increased shoot development, while dusts of 0·3% or 0·8% **I** had no effect. 0·1% **I** had no effect on shoot development of Midland Bermuda grass. Coastal Bermuda grass did not respond to any level of **I**. Rooting of Coastal Bermuda grass was improved by all levels of **I**, whilst that of Suwanee was improved only by the 0·3% and 0·8% levels. A. H. CORNFIELD.

Effect of source and rate of nitrogen on bromegrass. W. M. Laughlin (*Agron. J.*, 1963, **55,** 60—72).—NH_4NO_3, $(NH_4)_2SO_4$ and $Ca(NO_3)_2$ were equally effective as N sources for bromegrass; urea was not usually as effective, whilst anhyd. NH_3 and $CaCN_2$ were inferior. Split N applications spread forage yields over the season, but total seasonal yields were no greater than when a single application was made in the spring. Forage yields were usually higher when N was applied in the spring than in the autumn. A. H. CORNFIELD.

Effect of soil temperature and nitrogen fertilisation on the growth of soft chess, *Bromus mollis*, at two elevations. M. B. Jones, C. M. McKell and S. S. Winans (*Agron. J.*, 1963, **55,** 44—46).—Application of N increased growth of soft chess very little when the average soil temp. was <7·2°. The greatest response to N application occurred when the soil temp. was between 8·3° and 12·8°. Response to N decreased when soil temp. was >12·8°. A. H. CORNFIELD.

Effect of nitrogen rate and clipping frequency on yield of Pensacola Bahiagrass. E. R. Beaty, J. D. Powell, R. H. Brown and W. J. Ethredge (*Agron. J.*, 1963, **55,** 3—4).—Forage production of Pensacola Bahiagrass on a loamy sand increased with rate of application of N up to 200 lb./acre. Clipping every 6 weeks resulted in greater forage yields than did clipping at shorter intervals. A. H. CORNFIELD.

Effect of extent of splitting nitrogen fertiliser application on Pangola grass. L. R. Brenes (*J. Agric. Puerto Rico*, 1962, **46,** 171—174).—The carrying capacity for dairy and beef cows of Pangola grass on a clay receiving 360 lb. N as $(NH_4)_2SO_4$ per acre split into 3 or 4 applications (applied every 4 or 3 months) was compared with that of a Para grass–kudzu pasture receiving no N. There were no significant differences in animal performance between any of the treatments. A. H. CORNFIELD.

Chemical changes and respiratory drift during the air drying of ryegrass. J. F. Melvin and B. Simpson (*J. Sci. Fd Agric.*, 1963, **14,** 228—234).—Respiration continues throughout most of the drying period, but at a progressively slower rate. With regard to losses, fructosans and the total sol. fructose residues decreased almost continuously throughout the drying period. Sucrose contents decreased initially then increased. Most of the actual losses of dry matter determined by weighing were caused by loss of sol. carbohydrate, but some protein breakdown was observed. Except at the beginning of the drying process, the changes in composition did not account for the CO_2 production. (22 references.) E. M. J.

Distribution of major and trace elements in common pasture species. G. A. Fleming (*J. Sci. Fd Agric.*, 1963, **14,** 203—208).—Analytical results are presented for 20 elements in different parts of four common grasses and one clover. A diversity in distribution pattern for different elements in heads, leaves and stems is evident. Mg shows a similar pattern for all species, being highest in leaves and lowest in stems. N distribution parallels that of Mg in some cases, but not in others. With meadow fescue and perennial ryegrass the N contents of heads and leaves are similar. The effect of these findings on more specific problems of nutrition are considered, e.g., to what extent the low Mg contents in grass stems is of importance in the assimilation of Mg by grazing stock at times when clover growth in pastures is poor or at a standstill. (18 references.) E. M. J.

Comparative responses to copper of some tropical and temperate pasture legumes. C. S. Andrew and P. M. Thorne (*Aust. J. agric. Res.*, 1962, **13,** 821—835).—Of the ten pasture legumes examined, *Trifolium alexandrinum* and *Stylosanthes bojeri* were the most sensitive to Cu deficiency in soil. With Cu <4 p.p.m. in the material plants may be considered to be deficient but the occurrence of a higher Cu content does not always indicate a satisfactory availability of Cu in soil. The visual symptoms of Cu deficiency differ within the species. (35 references.) W. ELSTOW.

Effect of management practices on yield and composition of three grass–legume mixtures. O. J. Hunt and R. E. Wagner (*Agron. J.*, 1963, **55,** 13—16).—The height to which the forage was cut had the greatest influence on forage yields and botanical composition of ladino clover in combination with orchardgrass, tall fescue and bromegrass. Cutting to 2 in. produced more forage with a greater % of clover than did cutting to 4 in. During the first 2 years less frequent cutting (3—4 cuts per year) produced more forage than did more frequent cutting (4—5 cuts per year). As the clover content of the less frequently cut plots began to decrease yields were reduced to less than those on the frequently cut plots. Yields generally increased as the date of the first cut was delayed from 20 April to 1 June, particularly on frequently cut plots cut to the 2-in. height. Grasses generally increased in the mixture when cutting was infrequent and at the 4 in. height. The contribution of the grass to forage yields decreased in the order tall fescue, orchardgrass, smooth bromegrass. Tall fescue prevented the ingress of weeds to a greater extent than did the other grasses. A. H. CORNFIELD.

Effect of phosphorus and potassium fertilisers on legume composition of grass–legume mixtures. O. J. Hunt and R. E. Wagner (*Agron. J.*, 1963, **55,** 16—19).—Application of K to a silt loam greatly increased the yields of legumes and the proportion of legumes in grass–legume mixtures. The effect was greater with high than with low lime application for ladino clover and Kentucky bluegrass, but the reverse was true for birdsfoot trefoil. The greatest increase in legume yields due to K application was with ladino clover growing with tall fescue. P applications had little effect on the performance of the mixtures. All mixtures were weedier with low than with high lime. A. H. CORNFIELD.

Influence of a legume on soil fertility under a grazed tropical pasture. A. W. Moore (*Emp. J. exp. Agric.*, 1962, **30,** 239—248).—Inclusion of centro (*Centrosema pubescens*) in a giant stargrass (*Cynodon plectostachyus*) pasture on a latosol resulted, after 2 years, in significantly higher levels of org. matter, total N and nitrifiable N (2 weeks incubation at 35°) in comparison with the grass alone. The legume increased soil N to the 1 ft. depth by 250 lb./acre per annum. Inclusion of the legume had no effect on available P, pH or C/N ratio. The legume increased the N% of the associated grass from 1·8 to 2·4, but had no effect on P, K, Ca or Mg contents of the grass. A. H. CORNFIELD.

Phosphate manuring of legumes. IX. Direct and indirect manuring of cereals in rotation with *rabi* legumes. S. Sen, S. S. Bains and B. P. Mathur (*J. Indian Soc. Soil Sci.*, 1962, **10,** 283—288).—Growth of fodder legumes, methra (*Trigonella faenum-graecum*), senji (*Melilotus parviflora*) and berseem (*Trifolium alexandrinum*) maintained soil fertility much better than did pulses, peas or gram. All except gram

responded to P fertilisers and in most cases addition of N enhanced the effect. Inclusion of fodder legumes in a 2-year rotation (legume–maize–wheat–fallow), with N + P given to the legume, increased cereal yields but to a smaller extent than did direct application to the cereal crop. A. G. POLLARD.

Effect of environmental factors on the growth of lucerne in the field. G. Stanhill (*Neth. J. agric. Sci.*, 1962, **10,** 247—253).—The measured dry-matter production of a frequently-irrigated and heavily-fertilised field crop of lucerne was compared with the potential photosynthesis calculated from measurements of solar radiation by De Wit's method (*ibid.*, 1959, **7,** 141). The calculated amounts agreed well with measured dry-matter production after corrections had been applied for losses due to respiration, root growth, and light wasted beneath the crop canopy. A. H. CORNFIELD.

Establishment and yield of late-summer lucerne seedings as influenced by placement of seed and phosphate fertiliser, seeding rate and row spacing. S. G. Carmer and J. A. Jackobs (*Agron. J.*, 1963, **55,** 28—30).—Lucerne yields in the first harvest year from late-summer seedings were better when the P fertiliser was banded with the seed than when it was broadcast, only when emergence was delayed by dry weather; under favourable weather conditions there were no differences in yields between banded and broadcast P. Total yields per acre and plant size tended to increase with seed rate from 4 to 8 lb. but to decrease with 12 lb./acre. Yields also increased with decreasing row spacing (8 in. to 4 in.). A. H. CORNFIELD.

Effect of amino-acids on germination of red clover seeds. W. A. Kendall (*Agron. J.*, 1963, **55,** 51—52).—Eighteen seed lots representing six cultivars of red clover were germinated on substrates containing six different amino-acids. Although there were significant differences in germination between seed lots and between amino-acids, the differences were not sufficiently consistent to be useful of identifying cultivars. A. H. CORNFIELD.

Response of certain *Trifolium* spp. to calcium in sand culture. R. W. Snaydon (*Plant & Soil*, 1962, **16,** 381—388).—*Trifolium incarnatum* showed the greatest response in dry-matter yields to increasing concn. of Ca (4—128 p.p.m.) in the nutrient solution, whilst *T. hybridum* showed no significant response to increasing concn. of Ca. *T. pratense* and *T. repens* gave intermediate results. There was a marked relationship between response to Ca and edaphic tolerance of the species. A. H. CORNFIELD.

Chemical fruit thinners. R. Schumacher (*Schweiz. Landw. Forsch.*, 1962, **1,** 23—46).—The effects of naphthyl-acetic acid and -acetamide prep. as fruit thinners are studied on different varieties of apples. The amide prep. are more effective and cause less leaf damage. Some varieties of apple, viz., Jonathan and Golden Delicious, are easier than others to thin. All chemical thinners accelerate ripening and diminish storing time. Spraying should take place one week after petal fall, preferably at 10—15°. (13 references.) J. V. RUSSO.

Effect of sugar-cane filter-press cake on growth of tomato plants. M. del C. C. Fernández (*J. Agric. Puerto Rico*, 1962, **46,** 167—170).—Application of 1% sugar-cane filter-press cake to sterilised soil increased the growth of tomato plants in pot tests and accelerated flowering. Application of the material to unsterilised soil had no effect on growth of the plants. A. H. CORNFIELD.

Fertiliser placement for kale. P. F. Ryan (*Irish J. agric. Res.*, 1962, **1,** 231—236).—On an acid soil, deficient in P, placement of superphosphate or of a mixed fertiliser (N 8, P 8, K 13%) for kale, 2 in. below the seed in 9-in. rows gave much greater yields than did broadcast applications. The benefit of placement was associated more particularly with the P than with the K or N components of the fertiliser. Similar trends were observed on more productive soils although differences due to methods of application were much smaller. A. G. POLLARD.

Sulphuric acid treatment of spinach seed. G. D. Reading, J. L. Blowers and M. J. Goode (*Ark. Farm Res.*, 1962, **11,** No. 4, 12).—Spinach seed soaked in H_2SO_4 (conc. technical grade) for 5 min., washed in running water for 15 min. and then air-dried for 1—2 weeks showed accelerated and increased germination and improved plant vigour. Seed-borne *Colletotrichum spinaciae* was eliminated. Acid treatment for 20 min. markedly lowered germination of the seed. A. G. POLLARD.

Micro-nutrient deficiencies in arable crops in Romney Marsh. II. Manganese deficiency in peas and cereals. T. H. Rose and W. Dermott (*Emp. J. exp. Agric.*, 1962, **30,** 263—275).—On these soils of pH 7·4—8·1 yields of peas were reduced seriously when Mn deficiency was severe. Spraying with $MnSO_4$ during flowering gave only slight increases in yields; spraying before flowering was necessary for full control. Soil application of $MnSO_4$ + superphosphate gave good yield increases and some control of marsh spot; $MnSO_4$ alone was not as effective. EDTA, Mn-EDTA and Mn-frits applied to the soil were relatively ineffective. Application of superphosphate to cereals aggravated Mn deficiency. Multiple Mn sprays or $MnSO_4$ + superphosphate applied to the soil gave the largest increases in cereal yields. Practical recommendations for control of Mn deficiency are given. A. H. CORNFIELD.

Pod and seed development in canning peas as influenced by mineral nutrition and root temperature. G. R. Klacan (*Dissert. Abstr.*, 1962, **23,** 782).—The effects of various essential elements on reproductive development in peas in nutrient solution cultures were studied. Increase in K supply tended to increase the no. of seeds per pod. In field tests with P fertiliser the best results were obtained when the fertiliser was banded 2 in. to the side and 2 in. below the seed and when the rows were placed 9 in. apart. Root temp. between 62 and 74°F were the most favourable. F. C. SUTTON.

Influence of mineral nutrition on the growth and chemical composition of *Asparagus officinalis*. L. D. Brown (*Dissert. Abstr.*, 1962, **23,** 1475—1476).—A series of studies were initiated to determine the current nutritional status of *A. officinalis* in terms of its mineral composition and thus its requirement for the utilisation of applied fertilisers. Experiments were also designed to ascertain if variable fertilisation can alter significantly the composition of the plant and the marketable yields obtained. Data collected from thirty commercial asparagus growers are given. F. C. SUTTON.

Use of foliar analysis in control of nutrition of carnation. D. Blanc (*C. R. Acad. Agric. Fr.*, 1962, **48,** 791—797).—Reliable indications of the nutritional state of the plants can be obtained from analyses of leaves taken from the middle of the flowering stem after the development of 5—6 pairs of leaves, and when the flower buds are beginning to show colour. Optimum yields are obtained at 3·9—4·3% of foliar N (dry basis), and at ratios of foliar K_2O/N = 1—1·2. A ratio of P_2O_5/N of ~0·25 is satisfactory. P. S. ARUP.

Response of sugar-cane to fertiliser application in relation to soil types in the Jagadhari factory zone. J. M. Sharma and S. S. Sani (*J. Indian Soc. Soil Sci.*, 1962, **10,** 249—253).—Manurial trials on typical sugar-cane soils are recorded. Cane yields showed general but varied response to N and in some cases to P. A. G. POLLARD.

Sulphur deficiency in sugar-cane. A. K. Dutt (*Emp. J. exp. Agric.*, 1962, **30,** 257–262).—Chlorosis and anthocyanin pigment formation in sugar-cane leaves was cured by foliar application of 0·1% $ZnSO_4,7H_2O$ or $FeSO_4,7H_2O$ or 0·001N-H_2SO_4, and also by irrigation of the plant. A. H. CORNFIELD.

Losses of sucrose in cut cane kept under shade or sun for different periods. E. Boneta-Garcia and M. A. Lugo-López (*J. Agric. Puerto Rico*, 1962, **46,** 189—194).—There were significant losses of sugar with time of storage of the cut cane of six varieties, with similar differences in rate of losses whether storage was in the sun or shade. There were varietal differences in the extent of loss of sugar during storage of the cut cane. A. H. CORNFIELD.

Quality of Gezira cotton. I. Relationship between quality and crop earliness. J. E. Jackson and R. C. Faulkner. **II. Effect of site, nitrogen and spacing on seed-cotton grade.** J. E. Jackson, R. C. Faulkner and D. K. Dutta Roy (*Emp. J. exp. Agric.*, 1962, **30,** 192—206, 207—214).—I. The poor quality of late picks of cotton as compared with early picks is not merely a senescence effect but is due to adverse environmental conditions late in the season. Crop earliness was of less importance in controlling the average quality of an early than of a later variety of cotton. For both varieties the relationship was strongest in the location where the crop normally matures late. Preliminary data are presented suggesting an important relationship between temp. during the period of lint development and the quality of the mature cotton.

II. Differences in grade of cotton at various sites was shown to be largely a result of differences in crop earliness. Application of N, by increasing the yield of the poor-quality late picks, depressed the average grade, though it did not lead to a correspondingly great reduction in the yields of the better grades. Close spacing, which leads to the production of an earlier crop, had a favourable effect on the yields of the medium grades. A. H. CORNFIELD.

Seasonal nitrate content of cotton petioles as affected by nitrogen application and its relationship to yield. A. J. MacKenzie, W. F. Spencer, K. R. Stockinger and B. A. Krantz (*Agron. J.*, 1963, **55,** 55—59).—Petiole NO_3^--N in cotton during the growing season was proportional to the rate of N fertiliser applied (60—300 lb./acre). Petiole NO_3^-N levels of 16,000, 8000 and 2000 p.p.m. (dry basis) during the early-, mid- and late-bloom stages respectively were adequate for high cotton yields. Moisture and variety effects on petiole NO_3^- were small compared with the effects of N fertilisers. A. H. CORNFIELD.

Amino-acids in root exudates of cotton. C. B. Sulochana (*Plant & Soil*, 1962, **16**, 312—326).—From 7 to 11 amino-acids (**I**) were found in the root exudates of cotton. Seven were found in both *Fusarium* wilt-free and wilt-sick soils, the amounts being higher in the latter soil. Diploid cotton strains exuded greater amounts of **I** than did amphidiploid strains. More **I** were exuded from plants grown on wilt-sick than on wilt-free soil, except for one diploid strain.
A. H. CORNFIELD.

Cotton roots and vitamin-requiring and amino-acid-requiring bacteria. C. B. Sulochana (*Plant & Soil*, 1962, **16**, 335—346).—The influence of cotton roots on amino-acid-requiring bacteria was greater than on vitamin-requiring bacteria. The rhizosphere effect of diploid cotton strains was greater than that of amphidiploid strains. The rhizosphere effect of plants inoculated with *Fusarium* was lower than that of controls; the effects of apparently healthy diploid plants and those of inoculated amphidiploid plants in wilt-sick soil were higher than that of controls. A. H. CORNFIELD.

Influence of soil and irrigation water on chemical composition and quality of cigar tobacco. C. K. R. Kurup, A. S. Sastry and D. S. Rao (*J. Indian Soc. Soil Sci.*, 1962, **10**, 237—242; cf. *ibid.*, p. 10).—The yield, composition (K, Ca, Mg, P, Cl, N, nicotine) and leaf burn of tobacco was influenced more by the nature of the irrigation water than by soil characteristics. A. G. POLLARD.

Manganese toxicity in Burley tobacco. A. J. Hiatt and J. L. Ragland (*Agron. J.*, 1963, **55**, 47—49).—Tobacco grown in solution culture showed characteristic toxicity symptoms (chlorosis and necrotic spotting) when tissue Mn concn. reached 3000 p.p.m. (dry basis) although growth was not reduced even with 5000 p.p.m. Mn in the tissue. Reduced growth occurred when Al in the tissue exceeded about 200 p.p.m., but no characteristic Al-toxicity symptoms occurred. Injury from high concn. of Mn in the nutrient was largely overcome by increasing the Fe concn. of the solution, which also reduced absorption of Mn by the plant. Mn-toxicity symptoms were different from Fe-deficiency symptoms.
A. H. CORNFIELD.

Influence of potassium on tobacco yields and quality on a Mabí clay. G. Samuels, F. González-Vélez, E. G. Boneta-García and A. Sierra-Brecero (*J. Agric. Puerto Rico*, 1962, **46**, 183—188).—Application of 100—200 lb. of K_2O/acre had no effect on, whilst 300 lb./acre significantly increased, yields of tobacco on a clay (pH 5·6). The application of 200 lb. of K_2O + 2 tons of CaO/acre also resulted in significantly increased yields. The last-named treatment also produced the highest quality of tobacco and resulted in the best financial returns. A. H. CORNFIELD.

Fermentation of Maryland tobacco grown under different rates of nitrogen fertiliser. D. D. Tyrer (*Dissert. Abstr.*, 1962, **23**, 1508).—Samples of shredded tobacco (50 g. dry wt.) were fermented at 30% moisture in Dewar flasks in an incubator, and under constant aeration. Provision was made for the measurement of thermogenesis, the evolution of CO_2 and NH_3 from the sample. Fermentation was terminated when the CO_2 yield was 0·04—0·07 ml. per g. of tobacco per h. In general, there was a close parallelism between the trends for thermogenesis and the production of CO_2 in relation to the treatments. F. C. SUTTON.

Simple plant phenolic compounds in tobacco flowers and leaves. R. Watanabe (*Dissert. Abstr.*, 1962, **23**, 1509).—Knowledge of the distribution of certain polyphenolic compounds in each floral part of the one-sucker tobacco, *Nicotiana tabacum* L., could aid future biochemical, physiological and genetical investigations of tobacco. The techniques employed for isolating the polyphenolic compounds are described. The results of this study indicated that aesculetin and chlorogenic acid were present in four parts of the flower. Rutin and scopolin were present in the stamen, corolla and calyx but not in the pistils. The calyx was the only floral appendage of the four types studied which contained methyl ether deriv. of quercetin.
F. C. SUTTON.

Causes of loss of yield in groundnuts in the Sudan Central Rainlands. P. K. S. Clinton (*Emp. J. exp. Agric.*, 1962, **30**, 137—144).—Difficulties of establishing high populations of groundnuts in a newly opened area of the Sudan Central Rainlands were traced to pests, mainly soil-borne, which attacked the plant from the pre-emergence to the pod stage. The rôle of the various fungi and insects involved is discussed. A. H. CORNFIELD.

Intercropping. II. Castor-bean with groundnuts or soya-bean. A. C. Evans and A. Sreedharan (*E. Afr. agric. For. J.*, 1962, **28**, 7—8).—Intercropping castor-bean with soya-beans or groundnuts usually resulted in an overall gain in production per acre and never in an overall loss. Since insect attacks often reduce castor-bean yields drastically, intercropping will give a compensatingly high yield of the other crop. A. H. CORNFIELD.

Cobamide coenzyme contents of soya-bean nodules and nitrogen-fixing bacteria in relation to physiological conditions. M. Kliewer and H. J. Evans (*Plant Physiol.*, 1963, **38**, 99—104).—Soya-bean nodules showed a gradual increase in vitamin B_{12} coenzyme (**I**) content with age until flowering when concn. decreased slightly as the plants reached maturity. There were wide variations in coenzyme content of five *Rhizobium* species tested but very small differences between effective strains of the same species. The **I** content of *R. meliloti* and *R. japonicum* cells was markedly increased with increasing Co concn. in the culture medium. In the former the **I** has been isolated and identified as 5,6-dimethylbenzimidazolyl-cobamide (*ibid*, 1963, **38**, 55). *Azotobacter vinelandii* required an extremely small amount of Co for normal growth.
E. G. BRICKELL.

Physiological investigations into the white Lisbon yam, *Dioscorea alata* L. I. Breaking the rest period of tubers by chemical means, II. Growth period and out-of-season production. J. S. Campbell, V. O. Chukwueke, F. A. Teriba and H. V. S. Ho-A-Shu (*Emp. J. exp. Agric.*, 1962, **30**, 108—114, 232—238).—I. Ethylene chlorohydrin (**I**) was the most effective of a no. of chemicals tested for breaking the rest period of the white Lisbon yam. The rest period (normally about 4 months) could be broken by using successively lower concn. of **I** the closer the period of natural sprouting; thus a dip in 8% **I** was necessary 4—6 weeks after harvesting the roots, but 4% **I** was sufficient with longer periods after harvesting. After 2—3 weeks there was 70—100% germination.

II. Edible maturity of white Lisbon yams, following termination of the normal resting period by treatment with ethylene chlorohydrin, was reached in 7·5—8 months after planting at any time from Jan. to June. Under Trinidad conditions supplementary irrigation was necessary. Both yields and tuber quality were reduced by application of 2,4-D for stimulating early maturity by inducing vine senescence. A. H. CORNFIELD.

Leaf analysis of Lacatan bananas as guide to the nutrition of the plant. C. W. Hewitt and R. E. Osborne (*Emp. J. exp. Agric.*, 1962, **30**, 249—256).—For adequate yields of bananas the min. leaf dry-matter levels of the major nutrients were for N 2·6%, for P_2O_5 0·45% and for K_2O 4·0%. Splitting the N applications into 2—6 applications per annum had little effect on leaf N %, but highest banana yields were obtained when the application was split into three. K applications had the most profound effect on yields as well as on leaf K %. When the leaf contained 2·5% of K_2O application of 4 cwt. of KCl doubled the yields. A. H. CORNFIELD.

Experiments on plantain production with conservation in the mountain region of Puerto Rico. J. Vicente-Chandler and J. Figarella (*J. Agric. Puerto Rico*, 1962, **46**, 226—236).—Yields and quality of plantain on a sloping latosol were as good by planting directly in to the sod, followed by strip cultivation for erosion control, as after clean cultivation. Optimum fruit yields (5 tons of edible pulp per acre) were obtained with 800 trees per acre. Yields were increased by application of 200 lb. each of N and P_2O_5 per acre but not by lime, Mg or K. Relationships between fruit yields and leaf nutrient % are presented. A. H. CORNFIELD.

Relationship between crop growth rate and leaf area index in the oil palm. A. R. Rees (*Nature, Lond.*, 1963, **197**, 63—64).—The net assimilation rates of oil palms has been determined in a nursery oil-palm spacing experiment; the optimum leaf area index was about 3. The implications of the results to increasing crop production in plantations are discussed. (11 references.) S. A. BROOKS.

Increased supply of soil nitrogen brought about by *Pinus*. B. N. Richards (*Ecology*, 1962, **43**, 538—541).—In lateritic podsolic soils of eastern Australia, growth of hoop pine (*Araucaria cunninghamii*) is restricted by N deficiency. When planted under 5—6-year stands of loblolly pine (*Pinus taeda*) or slash pine (*P. elliottii*) growth of hoop pine was greatly improved. Pre-cropping with legumes produced similar results. The mechanism by which *Pinus* provides the additional N supply is discussed. A. G. POLLARD.

Leaf analysis as a guide to the nutrition of *Hevea brasiliensis*. V. Leaf sampling technique for mature trees. V. M. Shorrocks (*J. Rubb. Res. Inst. Malaya*, 1962, **17**, 167—190).—The effects of applications of $(NH_4)_2SO_4$, KCl, rock phosphate, $MnSO_4$ and $CuSO_4$ on the nutrient composition of the leaves of *H. brasiliensis* were discussed. Three types of leaves were considered and the most generally satisfactory for sampling were shaded leaves from low branches; 18 tables of results are given and statistically evaluated.
J. L. WALPOLE.

Pest Control

Pesticides and wildlife, the conservationist's point of view. N. W. Moore (*Chem. & Ind.*, 1962, 2130—2131).—The limiting factors of a

species in its ecosystem and the need to recognise the value of the existing habitat are stressed. The effects of org. P and chlorinated hydrocarbon insecticides on birds are given and suggestions for future research on this problem are made. E. C. DOLTON.

Synthesis and antifungal evaluation of some derivatives of dialkoxybenzoic and dialkoxycinnamic acids. A. K. Kapadia and G. A. Wiese (*J. pharm. Sci.*, 1962, **51**, 962—970).—A no. (24) of dialkylaminoalkyl esters (alkyl is Me or Et) of 3,4-dialkoxy-benzoic acid and -cinnamic acid (4-alkoxy is OMe and 3-alkoxy is OMe or OEt) with Cl, Br or I at position 5 are prepared by interaction of the acid and the appropriate alkanol. The products had antifungal activity (to *Trichophyton mentagrophytes*, *T. rubrum* and *Microsporum gypseum*) comparable with that of griseofulvin and undecylenic acid. Most had no antifungal activity *in vitro*. (24 references.) B. H. COUPE.

Organo-insectofungicides. LXVI. Synthesis and insecticide features of esters of some carbamic acids. K. D. Shvetsova-Shilovskaya, N. N. Mel'nikov, Z. I. Maksimova, T. S. Zakharova and L. P. Bocharova (*Zh. obshch. Khim.*, 1962, **32**, 3230—3232).—As possible new insectofungicides, cyanoethyl and cyanoisopropyl esters of some arylcarbamic acids (**I**) were synthesised and also some aryl esters of alkylcarbamic acids (**II**). **I** were obtained by reaction of the corresponding aryl isocyanate with an alcohol. Esters of alkylcarbamic acid were prepared by reaction of aliphatic amines with esters of chloroformic acid. During insecticide tests it was observed that all esters of **I** were almost entirely inactive, but those of **II** were highly active, especially the *m*-tolyl ester of methylcarbamic acid (**III**) which was more toxic than Sevin. With larger *N*-substituents in the carbamic acids the activity of the compounds lessened. **III** was considered to be of practical interest. A. L. B.

Fungicides. VII Antifungal activity of certain hydroxy-nitroalkanes and related compounds. A. N. Bates, D. M. Spencer and R. L. Wain (*Ann. appl. Biol.*, 1963, **51**, 153—160).—Four series of aliphatic nitro-compounds were synthesised and examined for their fungistatic effects in mycelial growth tests. Those containing the ethylenic linkage were the most active. A. H. CORNFIELD.

Comparison of invertebrate populations of soil and litter of mown grasslands in areas treated and untreated with pesticides. E. F. Menhinick (*Ecology*, 1962, **43**, 556—561).—Differences in species distribution, in surface litter and in soil following applications of numerous pesticides are recorded. Destruction of certain predatory organisms is a probable cause of the changes observed. Disturbance of the natural balance by pesticides may lead to instability of species distribution. A. G. POLLARD.

Use of phorate and Di-syston for potato insect control and a study of factors which influence phorate absorption by plants and loss in the soil. R. S. Patterson (*Dissert. Abstr.*, 1962, **23**, 1471—1472).—The most effective means of applying systemic insecticides, phorate and Di-syston, for the control of insects feeding on the foliage of field potato plants was in granular formulations to the soil at the time of planting. A bio-assay technique with *Drosophila melanogaster* (Meig.) as the test insect was used to determine toxic residues. Very little of the phorate applied to the soils was absorbed by plants or leached out of the soil. F. C. SUTTON.

Effect of DDT on cone production, germination and seedling survival in the boreal forest. G. M. Woodwell (*Ecology*, 1962, **43**, 396—403).—Data obtained from forested areas in which DDT was used extensively in pest control (notably against spruce budworm) show that no ill-effects on cone formation or species distribution were attributable to the insecticide. Probably at least 10-fold commercial concn. would be needed to produce injurious effects on the trees. A. G. POLLARD.

Comparison of five extraction procedures for the recovery of DDT residues in field-treated collards. L. J. Hardin and C. T. Sarten (*J. Ass. off. agric. Chem., Wash.*, 1962, **45**, 988—990).—The procedures tested were : (*a*) tumbling with n-hexane, (*b*) blending and tumbling with n-hexane, (*c*) blending with isopropyl alcohol followed by tumbling, (*d*) blending with isopropyl alcohol and then with n-hexane (*e*) grinding with Na_2SO_4 and tumbling with n-hexane. Procedure (*a*) gave low results ; (*d*) is recommended, giving recoveries of 92—95%. A. A. ELDRIDGE.

Inhibition of *Hypoxylon pruinatum* by pyrocatechol isolated from bark of aspen. M. Hubbes (*Science*, 1962, **136**, 156).—The fungistatic action of a meal made by grinding the freshly gathered bark of various types of poplars has been determined. The action varied in intensity with the species of poplar, the bark of aspen (*Populus temuloides*) producing the greatest inhibitory effect. Inhibition was consistently greater in bark collected in the autumn and winter than in that obtained in the summer and greatest, also, in bark from the base of the trunk ; it varied with the locality of the tree. The effective agent appeared to be identical with pyrocatechol. T. G. MORRIS.

Action of metham-sodium in soil. I. Development of an analytical method for the determination of methyl isothiocyanate residues in soil. M. G. Ashley and B. L. Leigh. **II. Factors affecting the removal of methyl isothiocyanate residues.** M. G. Ashley, B. L. Leigh and L. S. Lloyd (*J. Sci. Fd Agric.*, 1963, **14**, 148—153, 153—161).—I. As a glasshouse soil fungicide metham-sodium is recommended for application as a 0·5% w/v solution. It breaks down to methyl isothiocyanate (**I**), the reaction being hastened, e.g., by Fe and Cu ions and retarded by Zn, Ca and Ni. A method is described for determining small amounts of **I** in soil. The i.r. absorption, at 4·7μ, of a CCl_4 extract of the soil is measured in a 5-mm. cell ; the method is sensitive to ~1 p.p.m. (10 references.)

II. The activity of **I** is both fungicidal and phytotoxic. Various factors affect the release of **I** from soil : (*a*) temp. is the most important, at 15° release can be 25—50% greater than at 10° ; (*b*) residues are released quicker from sandy or clay loams than from soils containing peat ; (*c*) by increase in pH of the soil and (*d*) from drier as compared with water-saturated soils. Release of **I** is mainly by volatilisation but decomposition is also brought about by microbial and possibly chemical action. E. M. J.

Retention of aqueous suspensions on leaf surfaces. S. B. Challen (*J. Pharm. Pharmacol.*, 1962, **14**, 707—714).—Leaves of *Rumex obtusifolia* (Dock) of different age were dipped vertically into a 1% lycopodium spore suspension with and without 0·01% Na lauryl sulphate (**I**). The leaves were placed lower surface downwards and the relative extents of wetting examined. Immediately on withdrawal from the suspension complete coverage was noted, but subsequent recession of the liquid film caused drifting of the particles. Addition of **I** reduced clumping of the spores. Similar tests were applied to the leaves of many other common plants (without **I**) ; the wettability of hairy leaves depended on the type and arrangement of the trichomes. Wettability and distribution of particles is readily achieved with an open trichome pattern. With a closed pattern, wettability is poor but spores are retained well. Cuticles roughened by ridges are only partially wettable. B. H. COUPE.

Insecticide resistance–susceptibility tests with German cockroaches, *Blattella germanica*, L., in Puerto Rico. J. R. Gorham (*J. Agric. Puerto Rico*, 1962, **46**, 219—225).—A local strain of the German cockroach was resistant to $C_8H_6Cl_8$, chlordane and dieldrin but was susceptible, in decreasing order of effectiveness, to Bayer 29493 [*OO*-dimethyl *O*-(4-methylthio-*m*-tolyl) phosphorothioate], diazinon, malathion + pyrethrum synergised with piperonyl butoxide, and malathion. A. H. CORNFIELD.

Acaricidal activity of organic polymers. H. E. Aller (*Dissert. Abstr.*, 1962, **23**, 1469).—Some 27 water-dispersible org. polymers were tested for acaricidal effectiveness against the two-spotted spider mite *Tetranychus telarius* (L.). Org. polymers are not efficient acaricides since their efficacy is limited to motile mite stages contacted by the liquid phase. However, polymers such as the cellulosics can rapidly reduce large mite populations to levels more easily contained by standard or polymeric acaricides. F. C. SUTTON.

Potato russet scab. M. De V. Harrison (*Dissert. Abstr.*, 1962, **23**, 1478).—A species of *Streptomyces* was isolated from 82% of the russet scab lesions on freshly harvested tubers by cutting 5—7 thin sections from such lesions and planting them on 3% water agar in Petri dishes. This method was effective for isolating from both russet and common scab. High soil moisture was most favourable for russet scab infection. Pentachloronitrobenzene (PCNB), and 3,3,4,4-tetrachlorotetrahydrothiophen-1, 1-dioxide (experimental compound DAC-649) significantly reduced russet scab infection in the field but not in the greenhouse. F. C. SUTTON.

Influence of chemical potato beetle insecticides on aphid predators and aphid populations. P. Bovey and W. Meier (*Schweiz. landw. Forsch.*, 1962, **1**, 5—22).—In laboratory and field studies insecticides based on Ca arsenate, Sevin, DDT and dieldrin were all toxic, to varying degrees, to aphid predators and may lead to increased aphid populations and thence to greater virus infections. DDT and dieldrin have less effect than Ca arsenate on aphid predators but their use should be kept to a min. (11 references.) J. V. RUSSO.

Control of *Gloeosporium* spp. in apple orchards. A. T. K. Corke (*Ann. appl. Biol.*, 1962, **50**, 735—747).—Application of three org. Hg fungicides, DNOC and Dichlorophen to apple trees during dormancy reduced the viability of conidia of *Gloeosporium perennans* passing in rainwater over the sprayed bark and suppressed spore production on the acervuli. Whilst the org. Hg treatments greatly reduced spore viability they were less effective than the other materials in reducing sporulation over a long period. A. H. CORNFIELD.

Strawberry insects. R. E. Campbell and E. A. Taylor (*U.S. Dep. Agric. Fmrs Bull.*, 1962, No. 2184, 20¦ pp.).—Aphids, mites, weevils and other pests are described, together with methods for their control by dusts and sprays. E. G. BRICKELL.

Control of soil-borne pests and diseases of groundnuts in the Sudan Central Rainlands. P. K. S. Clinton (*Emp. J. exp. Agric.*, 1962, **30,** 145—154).—Yields of groundnuts were greatly increased by soil applications of dieldrin (1·5 lb./acre) together with seed dressings of 0·1% of dieldrin and an org. Hg prep. (Hg 20 p.p.m.) or 1000 p.p.m. of thiram. A. H. CORNFIELD.

Factors affecting rhododendron bud blast and its control. P. J. Howell and R. K. S. Wood (*Ann. appl. Biol.*, 1962, **50,** 723—733).—Laboratory tests provided no conclusive evidence that the fungus *Pycnostysanus azaleae*, which causes rhododendron bud blast, enters the bud tissue through wounds produced by oviposition of the leaf hopper *Graphocephala coccinea*. In the field healthy buds enclosed in muslin to exclude the insect did not become infected, whilst buds enclosed with the fungus but not the insect showed very few infections. Most infections occurred when both insects and fungus were enclosed with the bud. The insects themselves may carry spores of the disease. Of a no. of treatments tested for control, colloidal S was the most effective on *Rhododendron* var. Prince of Wales and had no toxic effects. Bordeaux mixture and phenyl Hg acetate sprays showed some phytoxicity. A. H. CORNFIELD.

The Japanese beetle. W. E. Fleming (*U.S. Dep. Agric. Fmrs Bull.*, No. 2151, 16 pp.).—Habits of, and damage due to, *Popillia japonica* are described together with methods for the control of both grubs and beetles. E. G. BRICKELL.

Effects on subsequent generations after γ-irradiation of larvae of *Lyctus brunneus* (Steph.) (Coleoptera, Lyctidae). J. D. Bletchly (*Ann. appl. Biol.*, 1962, **50,** 661—667).—A single irradiation of 4000 r on larvae of the powder-post beetle resulted in reduced and delayed emergence of the first generation. The no. of beetles per female returned to normal in subsequent generations. The vigour of emerged beetles as judged by their average wt. did not appear to be much affected. Irradiation at a sublethal dosage thus seems unsuitable for controlling infestations by wood-boring insects. A. H. CORNFIELD.

Elm leaf beetle in Arkansas. L. O. Warren (*Ark. Farm Res.*, 1962, **11,** No. 4, 11).—Application of phosphamidon (1 pint), dimethoate (0·25 lb.) or Bayer 22408 (*OO*-diethyl *O*-naphthalimidophosphorothioate) (0·5 lb./100 gal.) gave satisfactory control of the beetle. A. G. POLLARD.

Relative susceptibility of weeds to auxin herbicides. J. R. Hay (*Canad. J. Bot.*, 1962, **40,** 1401—1409).—Stem sections of *Silene cucubalus*, *Galeopsis tetrahit* and *Phaseolus vulgaris* cultured in media containing inorg. salts, sucrose, agar and one of the auxin herbicides, 2-methyl-4-chloro- or 2,4-dichloro-phenoxyacetic acid or 2-(2,4,5-trichlorophenoxy)propionic acid showed a degree of root formation and growth proportional to the effectiveness of these herbicides in the field. The response to the herbicides diminished when the stem sections were taken from more mature plants. No correlation was found between the susceptibility of a weed and the amount of herbicide recovered from it. The relative susceptibility probably depends on the mol. structure of the herbicide and on the amount, duration and functional importance of the susceptible plant tissues at the time of treatment. (22 references.) J. L. WALPOLE.

[A] **Comparison between the effectiveness of some pre-emergence herbicides applied in planting strips of *Hevea brasiliensis* on a sandy soil.** [B] **Weed control with simazine in planting strips of *Hevea brasiliensis* and in leguminous cover plants.** P. Riepma Kzn (*J. Rubb. Res. Inst., Malaya*, 1962, **17,** 191—203, 204—219).—[A] The effectiveness of 12 pre-emergence herbicides applied to planting strips was compared using *Paspalum conjugatum* as the principal weed species. Simazine, alone, was used in all the trials and, compared with hand weeding, it extended the period of control by 11·5 and 14·4 weeks respectively when used at 5 and 10 lb./acre. Atrazine gave similar results and atraton, Prometone and Prometryne merit further investigation. In limited tests, Banvel D (2-methoxy-3,6-dichlorobenzoic acid) and neburon showed promise but Alipur (a mixture of substituted urea and carbamate compounds), Banvel T (2-methoxy-3,5,6-trichlorobenzoic acid), Shell WL 3379 (2,6-dichlorobenzonitrile) and dalapon were disappointing. Amitrole showed pre-emergence activity and enhanced the effect of simazine when mixed with it.

[B] When simazine (**I**) was applied as a pre-emergence herbicide at 5 and 10 lb./acre to planting strips, a negative correlation between the clay content of the soil and the period of weed control was established. The max. period of control beyond that obtained with hard weeding was only 3·2 weeks on a coastal clay soil and over 9 weeks on a sandy inland soil. **I** is of less value for inter-row application owing to the encroachment of weeds from the unsprayed drills. J. L. WALPOLE.

Effect of 2,4-dichlorophenoxyacetic acid on seedling development and uptake and distribution of calcium and phosphorus in barley. L. H. Smith (*Dissert. Abstr.*, 1962, **23,** 1152—1153).—The greatest effect of 2,4-D on the Ca and P content in barley occurred in the pre-emergence application with the least effect at the five-leaf stage, the three-leaf application being intermediate in response. It is suggested that 2,4-D directly affects the uptake and translocation mechanism in barley. F. C. SUTTON.

Substituted uracil herbicides. H. C. Bucha, W. E. Cupery, J. E. Harrod, H. M. Loux and L. M. Ellis (*Science*, 1962, **137,** 537—538).—The compounds examined have low mammalian toxicity. When applied at 2 lb./acre 5-bromo-3-isopropyl-6-methyluracil (**I**) gave 100% kill of crabgrass, sorghum, wildoats, nut sedge, mustard and bean. 3-Butyl-6-methyluracil (**II**) was toxic to fewer plants but showed selective toxicity. The lethal doses of **I** and **II** to male white rats by oral administration were approx. 7500 and 3400 mg. per kg. respectively. T. G. MORRIS.

Weed control in a pineapple plantation in Ivory Coast. A. Silvy (*Fruits, Paris*, 1962, **17,** 501—505).—Diuron and monuron gave good results and tended to change the natural balance of adventitious plants to the advantage of *Digitaria* and *Paspalum*. Simazine, however, was effective against the whole weed spectrum. W. ELSTOW.

Antibacterial agents. Beecham Research Laboratories Ltd. (B.P. 888,110, 21.10.60. U.S., 22.10.59).—Acid-stable penicillin deriv., suitable for use as antibacterial agents (active against penicillin-resistant strains), as nutrional supplements in animal feeds, agents for the treatment of mastitis, and for the treatment of infections in poultry and animals (including man), comprise penicillonic acids (and their salts) substituted in the 6-position by $NH{\cdot}CO{\cdot}CH_2{\cdot}CHXR$ (X is halogen ; R is H, cyclohexyl, cyclopentyl or alkyl). A typical product is *6-(β-bromopropionamido)penicillanic acid.* F. R. BASFORD.

***exo*Dicyclopentadiene dioxide.** Union Carbide Corp., Assees. of S. W. Tinsley and P. S. Starcher (B.P. 887,778, 12.10.59. U.S., 30.10.58).—*exo Dicyclopentadiene dioxide*, m.p. 39—42°, b.p. 119—121°/4 mm., useful as a soil fungicide and nematocide, is produced by heating *endo*dicyclopentadiene with HI (in water) at 100°, then further heating the resulting *di-iodo-dihydro*-*exodicyclopentadiene* (b.p. 120—136°/10 mm.) with boiling aq. alkali hydroxide, and epoxidising the exodicyclopentadiene. F. R. BASFORD.

Manganese-containing dithiocarbamate reaction products. Chemische Werke Albert (B.P. 888,289, 26.5.60. Ger., 31.7.59 and 11.2.60).—NH_4 ethylene-bis-dithiocarbamate is reacted with 1 mol. of Mn oxide (or a mixture of Mn oxides) in presence of water at 10—50°, to form a new Mn-containing fungicidal product. A product containing 28% of Mn (prepared with MnO) is lethal to spores of *Alternaria tenuis* at a concn. of 5 μg. per sq. cm. F. R. BASFORD.

Halogeno-bisulphite addition compounds. Norwich Pharmacal Co. (B.P. 888,288, 13.4.60. U.S., 17.4.59).—Compounds useful in the treatment of Panama disease (caused by *Fusarium cubense*) of bananas have the general formula $OH{\cdot}CR(CH_2X){\cdot}SO_3NH_4$, and are obtained by condensing $R{\cdot}CO{\cdot}CH_2X$ with NH_4HSO_3 (X is Cl or Br ; R is Me or CH_2X). One example is *NH_4 1,3-dichloro-2-hydroxypropane-2-sulphonate*, m.p. 79—83°. F. R. BASFORD.

ββ-Disubstituted-β-cyanoketones. Rohm & Haas Co. (B.P. 887,411, 23.5.58. U.S., 31.5.57).—Compounds with fungicidal action towards *Stemphylium sarcinaeforme* and *Monilinia fructicola* are compounds $CN{\cdot}CR_2{\cdot}CHR'{\cdot}COR$ (R is Me or Et ; R′ is H or as R) of a total of 7—9 C, made by interaction of HCN with $CR_2{:}CR'{\cdot}COR$ at 130—350°/25—100 p.s.i. in an inert polar org. solvent and alkali metal cyanide catalyst. H. S. R.

Thionophosphonic acid esters. Farbenfabriken Bayer A.-G. (B.P. 888,346, 5.1.60. Ger., 7.1.59).—Compounds useful as pesticides are claimed, which have the formula $OR(OR'){\cdot}PS{\cdot}R''$ (R is alkyl of 1—4 C ; R′ is Ph substituted by at least 1 NO_2 and/or halogen and optionally by alkyl of 1—4 C ; R″ is alkyl of 1—4 C optionally containing halogen). As an example, *Et p-nitrophenyl methylphosphonothionate*, b.p. 108°/0·01 mm., is prepared which is 100% lethal to spider mites at a concn. of 0·001%. F. R. BASFORD.

Haloformamidines. Farbenfabriken Bayer A.-G. (B.P. 888,646, 13.5.60. Ger., 16.5.59).—Compounds of the general formula $R{\cdot}N{:}CX{\cdot}NR'R''$ are claimed (R is aryl optionally substituted ; X is Cl or Br ; R′ and R″ are H, alkyl, or together with N form a heterocyclic 5- or 6-membered ring). Details are given for the

prep. of *chloro(phenylimino)* (*dimethylamino)methane*, b.p. 131—133°/12 mm. The products have herbicidal properties.
F. R. BASFORD.

Piricularin-containing compositions. K. Tamari and Hokko Chemical Industry Co. Ltd. (B.P. 887,347, 14.9.59. Jap., 20.9.58).—There is claimed a composition (liquid or solid) comprising piricularin and at least one Cu- or Fe salt, suitable for controlling plant diseases, especially rice blast. A typical fluid prep. contains piricularin (from mycelium of *Piricularia oryzae* Cavara) (0·1), polyhydroxyethylene ether of nonylphenol (15), $CuSO_4.5H_2O$(0·1—3), $Fe_2(SO_4)_3$ (0·15—4·6) and water (to 100 pt.) F. R. BASFORD.

Thiophosphoric acid esters. American Cyanamid Co. (B.P. 887,676, 23.6.59. U.S., 30.6.58).—Compounds claimed have the general formula OR(OR')·PX·S·CH_2R'' [R and R' are low-mol. alkyl; X is O or S; R'' is 6-oxo-1, 6-dihydropyridazin-1-yl radical optionally substituted in the 3-position by OH, halogen, Ph, low-mol. alkyl, OMe, OEt, OAc, O·PS(OMe)$_2$ or O·PS(OEt)$_2$, and in the 4- and/or 5-position by halogen, Ph, low-mol. alkyl, or in the 2 positions together by a fused benzo ring]. They have high insecticidal, acaricidal and nematodicidal properties, and are prepared by reacting a 1-(chloromethyl)pyridazone with OR(OR')·PX·SM (M is alkali metal). Details are given for the prep. of OO-*Et*$_2$ S-(4-*bromo*-5-*chloro*-6-*oxo*-1,6-*dihydropyridazinylmethyl*) *phosphorothiolothionate*.
F. R. BASFORD.

Esters of phosphorus acids and insecticidal compositions containing them. Farbenfabriken Bayer A.-G. (B.P. 884,613, 14.10.59. Ger., 17.10.58 and 9.5.59).—The esters have the general formula RR'·PX·S·CH_2·C_6H_4·SR'' (R and R' are alkyl or alkoxy of 1—4 C; R'' is alkyl of 1—4 C; X is O or S). As an example, details are given of the prep. of OO-*Me*$_2$ S-p-*methylthiophenylthiomethyl phosphorothiolothionate*. The products are useful also against caterpillars.
F. R. BASFORD.

Pesticidal composition. American Cyanamid Co. (B.P. 887,308, 23.1.59. U.S., 24.1.58).—There are claimed insecticidal and acaricidal compositions in which the active ingredient is a 2-hydroxydiaziridine-3-carboxylic acid lactone (sydnone) substituted in the 1-position by Ph, C_6H_4Cl, benzyl, cyclohexyl or dodecyl, and optionally in the 3-position by Me, Cl or Br. Thus, nasturtium infested by aphides is completely freed from the latter by spraying with a 0·1% solution of 1-phenyl-2-hydroxydiaziridine-3-carboxylic acid lactone (1-phenylsydnone) in 65% aq. acetone. F. R. BASFORD.

Vinyl phosphate insecticides. Allied Chemical Corp. (Inventors: E. E. Gilbert, J. A. Otto and E. J. Rumanowski) (B.P. 888,648, 3.6.60).—Compounds (OR)$_2$PO·O·CR':CHCl, useful as systemic insecticides, are obtained by interaction of R_3PO_4 with R'·CO·$CHCl_2$ (R is alkyl; R' is dihalogenophenyl). *Et*$_2$ 2-*chloro*-1-(2,5-*dichlorophenyl)vinyl phosphate*, an oil, is prepared. F. R. BASFORD.

Phosphorus-containing amino-triazole derivatives. N. V. Philips' Gloeilampen-Fabrieken (B.P. 888,686, 24.4.58. Neth., 27.4.57 and 15.2.58).—Compounds useful in combating noxious organisms, especially fungi and insects (spider mites) comprise 3-amino-4,1,2-triazoles substituted in the 1- or 2-position by PX(NRR')·NR''R''' and optionally in the 5-position by alkyl, cycloalkyl, aryl or aralkyl (optionally substituted), or by an olefinic group or by carbalkoxy (X is O or S; R—R''' are aliphatic radicals). Directions are given for the conventional prep. of the *bis*-(NN-*dimethylamido*)-*phosphoryl* derivative of 3-(*tetramethylphosphorodiamidyl*)*amino*-4,1,2-*triazole*, m.p. 136·5—138°.
F. R. BASFORD.

Pesticidal compositions. Imperial Chemical Industries Ltd. (Inventors: F. R. Bradbury and A. Campbell) (B.P. 887,342, 24.6.59).—A dry, pulverulent pesticidal composition (an aq. dispersion of which on spraying affords a protective layer of improved adhesion), comprises a pulverulent pesticide (Gammexane, DDT, dieldrin, aldrin, zineb, captan, *N-p*-tolyldichloromaleimide or Cu_2Cl_2) (5—50); a wetting agent (1—10); a thickening agent (e.g., a carbohydrate or a deriv. thereof) 1—10; and a hydrophilic inorg. diluent, e.g., a swelling bentonite (25—80%). If desired, there may also be present alkali (0·5) and water-insol. inorg. solid diluent (to 100%). The composition should form with 25 pt. of water a thixotropic structure which can be easily sprayed and which after 4 h. has a Bingham yield value of $\not<$25 dynes per sq. cm.
F. R. BASFORD.

Halogenated hydrouracils. Diamond Alkali Co., Assee of F. B. Slazak (B.P. 888,627, 21.8.59. U.S., 25.8.58).—Compounds useful, *inter alia*, as nematocidal, insecticidal and herbicidal agents, comprise hydrouracils (2,4-dioxohexahydropyrimidines) substituted in the 1- and/or 3-position by Cl and optionally in the 5- and/or 6-position by halogen, alkyl or aryl. Details are given of the prep. of 1,3-*dichloro*-6-*methyl*-2,4-*dioxohexahydropyrimidine*, m.p. 87—87·5°, which contains 72% of available Cl and at concn. of 500 p.p.m. inhibits 90% of the growth of *Xanthomonas phaseoli*, and which causes no plant injury. H. S. R.

Herbicidal compositions. Badische Anilin- u. Soda-Fabrik A.-G. (Inventor: A. Fischer) (B.P. 875,048, 10.11.59. Ger., 11.11.58. Addn. to B.P. 849,794; J.S.F.A. Abstracts, 1962, i, 20).—There is claimed a synergistic herbicidal composition comprising a phenylurea, viz., NMeR·CO·NR'R'' (R is Me or OMe; R' is H, Me, hydroxyethyl or 2-hydroxypropyl; R'' is Ph or methyl-, di- or tri-methyl-, ethyl-, di- or tri-ethyl-, isopropyl-, di- or tri-isopropylphenyl) and a phenylcarbamate, viz., NHX·CO_2Z (X is Ph optionally substituted by Cl or Me; Z is alkyl or alkenyl of 1—4 C), also suitable carrier and dispersing agent. A preferred composition consists of *N*-β-hydroxypropyl-*N*-*m*-tolyl-*N'N'*-dimethylurea and butyn-3-yl chlorophenylcarbamate.
F. R. BASFORD.

Animal Husbandry

Dairy husbandry. A. S. Foot (*J. R. agric. Soc.*, 1962, **123,** 168—180).—Recent investigations on the nutrition of dairy cattle, the composition of milk, grazing and milking practices and on mastitis infection are reviewed. (38 references.) A. G. POLLARD.

Feeding of livestock. J. L. Corbett (*J. R. agric Soc.*, 1962, **123,** 157—167).—A review. Recent investigations on the protein feeding of ruminants, pigs and poultry and on the energy maintenance requirement of sheep are discussed. (44 references.)
A. G. POLLARD.

Digestive system of the ruminant. A. T. Phillipson (*J. R. agric. Soc.*, 1962, **123,** 89—98).—A summary of the chemical and biological processes involved in ruminant digestion and their relation to the value of feeding-stuffs. A. G. POLLARD.

Progress in assessing the energy value of feeding-stuffs for ruminants. K. L. Blaxter (*J. R. agric. Soc.*, 1962, **123,** 7—21).—The nutrient value of a feeding-stuff may vary considerably with the nature of the ration of which it forms a part. The preferred system of evaluation in which the units 'starch equivalent' and 'total digestible nutrients' are superseded by the metabolisable energy per lb. of food, measured at maintenance level, is discussed and corrections for the heat produced in the fermentation process yielding methane during digestion and for the plane of nutrition of the ration are explained. Application of the system to the prediction of food efficiency in growing, fattening or lactating cattle is shown.
A. G. POLLARD.

Composition of crude fibre and its influence on functions of the alimentary canal. A. Szczygiel (*Nahrung*, 1962, **6,** 701—707).—Published data are reviewed and discussed. The need for clearer conceptions as to the nature of crude fibre and for standardised experimental methods is pointed out. (12 references.)
P. S. ARUP.

Indirect methods of determining feed digestibilities and herbage intakes of grazing animals. III. Nitrogen as faecal index indicator in the herbage intake determinations. B. N. Gupta, B. N. Majumdar and N. D. Kehar. **IV. Use of the double indicator technique in determining feed digestibilities under stall conditions.** B. N. Gupta and B. N. Majumdar (*Ann. Biochem.*, 1962, **22,** 105—112, 239—244).—III. Indoor digestion trials were conducted on *kumaoni* bullocks and buffalo calves fed *sarwala* grass (*Heteropogon contortus*), Russian rye and *banchari* (*Sorghum halepense*). From data presented it was observed that the faecal N is highly related to the herbage intake and its digestibility and can, therefore, be successfully used as the faecal index indicator for the determination of herbage indigestibility. (22 references.)

IV. Five digestibility trials were conducted with *kumaoni* bullocks and buffalo calves fed spear grass, *sarwala*, (*Heteropogon contortus*) at young and pre-flowering stages, Russian rye and *banchari* (*Sorghum halepense*), both at pre-flowering stage. Results showed that the double indicator technique, using Cr_2O_3 as index of faecal output and N as the index of indigestibility, is a useful and dependable one under stall conditions where the composition of the animal feed may be easily determined. (19 references.) M. O'LEARY.

Rice protein supplementation. M. C. Kik and L. Easterling (*Ark. Farm Res.*, 1962, **11,** No. 4, 11).—Addition of casein (up to 5% in the ration) as a partial replacement or as an addition to a ration containing 5·46% of protein as milled white or whole brown rice increased the growth of rats. Further addition of methionine, threonine or L-lysine had no beneficial results. A. G. POLLARD.

Digestibility and feeding value of tropical grasses and kudzu. N. D. Dijkstra and J. G. P. Dirven (*Neth. J. agric. Sci.*, 1962, **10,** 275—285).—Crude protein, digestible crude protein, crude fibre and starch equiv. value are given for elephant grass, lucuntu grass, Para grass, Coastal Bermuda grass, Guatemala grass and two samples of kudzu. From the results and those of other workers on tropical grasses, a very high positive correlation (r = 0·980) was found between crude protein and digestible crude protein in the org.

matter. There was also a significant negative correlation ($r = -0.692$) between starch equiv. and crude fibre in the org. matter. A. H. CORNFIELD.

Preservation of fresh forage in small containers. J. T. Sullivan, E. S. Titlar, J. H. Graham, T. V. Hershberger and D. G. Routley (*Agron. J.*, 1963, **55**, 80—81).—Propylene and ethylene oxides were usually satisfactory as preservatives for maintaining the quality of grass and legumes stored at room temp. in containers up to 1 gal. capacity. Lucerne stored for 50 days with propylene oxide was consumed by sheep without ill-effect. Variable results were obtained with Cl_2 and ClO_2; because of their reactivity they do not tend to diffuse through the mass. Na metabisulphite usually gave poor results. A. H. CORNFIELD.

Estimation of green silage and the influence of additives on the quality of prepared silo fodder in agricultural practice. W. Schoch (*Schweiz. landw. Forsch.*, 1962, **1**, 74—92).—Silage quality is inversely related to the butyric and acetic acid and NH_3 contents of the product. Addition of 4—5 l. of 5 % formic acid/100 kg. of herbage is effective in obtaining good silages. NaCl, potato meal, cooked and dried potatoes and barley meal are not suitable as silage additives. (28 references.) J. V. RUSSO.

Ensilage. II. Plant maturity effects in the ensilage of ryegrass and clover under laboratory conditions. G. W. Lanigan and V. R. Catchpoole (*Aust. J. agric. Res.*, 1962, **13**, 853—863).—To obtain a suitable silage the initial quick fall in pH due to lactic fermentation should be to <4 and not to 4·2 as is commonly quoted. With the three varieties of ryegrass investigated this condition was obtained if the grass was harvested at the first appearance of the heads. Earlier harvesting gave less satisfactory results. Incorporation of white clover in the silage had an adverse effect by increasing losses of dry matter in the effluent and by destroying lactic acid thereby allowing the pH of the silage to rise. The proportion of white clover that could be added without marked ill-effect increased with later harvesting. W. ELSTOW.

Effects of additives on silages made from different herbages. R. B. McCarrick (*Irish J. agric. Res.*, 1962, **1**, 267—282).—Various additives were compared in the prep. of silages of grass-clover and of lucerne. All additives diminished dry-matter losses during ensilage of young herbage. When mature herbage was used losses were generally smaller and less consistent. Among the additives tested formic acid was particularly effective in limiting dry-matter losses and producing good quality silage; NH_4HSO_4 maintained a satisfactory level of N in the product. A. G. POLLARD.

Procedure for determining gelling characteristics of molasses in the presence of phosphates. G. R. Weber and F. D. Miller (*J. Ass. off. agric. Chem., Wash.*, 1962, **45**, 916—918).—Two phosphate buffer solutions are specified, (*a*) pH 2·7, (*b*) pH 2·3, for use at 42°. Laboratory gelling in 20 min.—2 h. or 2—24 h. corresponded to field gelling in 1—3 days or 3—6 weeks, respectively. The test is used to avoid the use of molasses having gelling properties for blending with H_3PO_4 feed supplements. A. A. ELDRIDGE.

***Aspergillus flavus* and groundnut toxicity.** P. C. Spensley (*Nature, Lond.*, 1963, **197**, 31—32).—Brief mention is made of a report which deals with the problem of *Aspergillus flavus* mould growing on groundnuts and causing 'turkey X disease', and with means of identifying affected nuts or meal. Means of minimising mould growth and toxin levels are suggested. S. A. BROOKS.

Sodium content of dry fodder and grass samples. A. Hasler (*Schweiz. landw. Forsch.*, 1962, **1**, 60—73).—Sodium contents (dry wt. basis) of grass are 0·031—0·147%, of first cut hay 0·015—0·096% and of second cut hay, 0·017—0·127%. Dandelion and perennial ryegrass contain more Na than do bluegrass or timothy. Fertilising with K decreases the Na content in dandelions but does not affect that of bluegrass. (26 references.) J. V. RUSSO.

Hypomagnesaemia. D. M. McAleese (*Sci. Proc. R. Dublin Soc.*, 1962, Ser. A, **1**, 297—318).—Rats were fed rations containing various levels of Ca (0·2—0·8%) and Mg (20—510 p.p.m.) and maintained at 10 or 23°. Responses were measured in terms of gain in wt., blood-serum-Mg and bone ash Mg. The Mg requirements for max. gain in wt. (*W*), maintenance of normal serum-Mg (*S*) and bone-ash-Mg (*B*) were 115, 365 and 288 p.p.m., respectively, in the ration. These requirements were increased by a rise in dietary Ca levels. Environmental temp. had no effect on requirements for *W* or *S* but that for *B* increased with temp. Increase in dietary Mg was associated with diminution in Ca content of soft tissues (hearts, kidneys) and *vice versa*. In lambs acute Mg-deficiency was accompanied by serum-hypocalcaemia which was not attributable to lack of Ca or vitamin D. Dietary requirements of Mg for *B* and *S* were 0·13 and 0·059% respectively for lambs partially depleted of Mg; otherwise the 0·059% level met both requirements. High dietary K (to 5%) had no effect on levels of serum-Mg in either Mg-depleted or control lambs. The bearing of these results on the occurrence of grass tetany is discussed. A. G. POLLARD.

Feeding cows with magnesium biscuits in practice. A Kemp (*Landbouwvoorlichting*, 1963, **20**, 28—29).—Criticisms as to the efficacy of the treatment are probably due to under-dosing. The min. effective daily dose is 50 g. of MgO per cow. Samples (11) of the biscuits from four factories, 10 of which were stated to contain 5% of MgO, actually contained 1—3·9% of MgO (average 2·5%). Complaints of non-palatability are probably due to staleness. P. S. ARUP.

Veterinary uses of electrolytes. R. Seiden (*Mfg Chem.*, 1962, **33**, 308—310).—U.S. proprietary prep. which make K^+, Ca^{2+}, Mg^{2+} and HCO_3^- ions available in the food or drinking water of animals (by partial replacement of NaCl) are considered and the effect on litter size (in rats), carcass shrinkage (in cattle), wt. increase in calves and pigs, etc., is briefly symmarised. The rationale is indicated. (10 references.) C. V.

Detection and determination of 1,2-dimethyl-5-aminoimidazole. L. B. Colvin, R. Silvaramakrishnan and J. R. Couch (*Chemist-Analyst*, 1963, **52**, No. 1, 9—11).—1,2-Dimethyl-5-aminoimidazole (**I**) is believed to be a degradation product of 1,2-dimethyl-5-nitroimidazole (**II**) when it is given to turkeys in massive doses. **I** will give a stable red-orange colour, with an absorption max. at 495 mμ, when diazotised and coupled with *N*-(1-naphthyl)ethylenediamine. By reducing it to **I** with Zn and HCl immediately before use, **II** may be determined by this method. J. HALL.

Rôle of phytic phosphorus in ruminant metabolism. I. Distribution of total phosphorus and phytic phosphorus in various cattle feeds. II. Influence of season and stage of maturity on phytic and total phosphorus contents of cultivated grasses. III. Influence of progressive maturity and irrigation on the total and phytic phosphorus contents of some winter crops. P. N. Johri and N. D. Kehar (*Ann. Biochem.*, 1962, **22**, 225—230, 231—234, 533—538).—I. In general both phytic and total P contents of oil-cakes, brans, tree leaves and grasses were high. 60—90% of the total P of oil-cakes and brans was in the form of phytic P. P contents of husks, hulls and cereal straws were low. (29 references.)

II. Experiments with the grasses *Panicum maximum, Cenchrus ciliaris, Chloris goyana, Cynodon plectostachyum, Heteropogon contortus* and *Bhanchara* showed that total P was highest at the time of active growth and that it decreased as the plants reached maturity. Phytic P was low during the period of active growth and reached its max. at maturity. (11 references.)

III. Experiments with oats, wheat, linseed, barley, 'berseem' (*Trifolium alexandrium*) and lucerne indicated that although total and phytic P contents appeared to be higher in non-irrigated than in irrigated crops the results were not statistically significant at the 5% level. The total P content of the crops decreased while the phytic P content increased as the plants approached maturity, the results in this case being highly significant. Phytic P content of the plants decreased on the development of seeds, suggesting that some of the phytic P is transported from the plant to the seeds. (15 references.) M. O'LEARY.

Nutritive value of grassmeal for pigs. I. Digestibility and total digestible nutrient (TDN) content. II. Digestible energy value: its relationship to total digestible nutrients. M. J. Lawlor, M. F. Maguire and E. J. Sheehy (*Irish J. agric. Res.*, 1962, **1**, 295—300, 301—305).—I. Comparison is made of 'good' and 'poor' grassmeal (description based on protein and fibre contents) when fed to pigs at levels of 10—30% of the ration. Digestibility coeff. for crude protein, crude fibre and TDN were markedly higher for the better meal. All values tended to rise with advancing ages of the pigs.

II. The total digestible energy of the two meals calculated from the calorific value of the TDN and parallel data obtained by measurement in a bomb calorimeter added further detail of the difference in feeding value between the two grassmeals. A. G. POLLARD.

Strain differences in the protein requirement of laying hens. R. H. Harms and P. W. Waldroup (*Poultry Sci.*, 1962, **41**, 1985—1987).—When a diet containing 17% of protein was supplied, hens of one strain had a slightly higher rate of egg production than had another, but the reverse was true with 11—13% protein diets. A similar trend held for body wt. A. H. CORNFIELD.

Flavours in poultry rations. H. Fisher and H. M. Scott (*Poultry Sci.*, 1962, **41**, 1978—1979).—Addition of 16 different flavouring materials to the diet of chicks had no effect on wt. gains or feed efficiency to 4 weeks of age. Addition of a commercial flavour blend also had no effects. Equal amounts of the flavoured and unflavoured feed were consumed when chicks were given free choice of both. A. H. CORNFIELD.

Anhydrous dicalcium phosphate as a source of phosphorus in poult diets. W. C. Supplee (*Poultry Sci.*, 1962, **41**, 1984—1985).—Addition of reagent-grade $CaHPO_4$ to a purified diet to supply 0·8% P in the diet resulted in virtually complete mortality of poults after 18 days, the only survivor being hopelessly rachitic. Where $CaHPO_4,2H_2O$ (N.F. grade) was supplied to give the same level of P, growth was excellent and there was no mortality. A. H. CORNFIELD.

Fate of endogenous magnesium-28 in laying hens. H. M. Edwards, jun., D. Nugara and J. C. Driggers (*Poultry Sci.*, 1962, **41**, 1975—1976).—Eggs laid by hens within 24 h. of receiving injections of ^{28}Mg contained 44% of the Mg dose in the shell, 18% in the albumin, 0·4% in the yolk and 7% in the droppings. Eggs laid later contained less of the Mg dose, whilst the droppings contained more. Four days after dosing, 65—99% of the Mg had been excreted in the egg and droppings. A. H. CORNFIELD.

Relationship of vitamin B_{12} content to methionine biosynthesis in turkey poult liver homogenates. B. W. Langer, jun. and F. H. Kratzer (*Poultry Sci.*, 1962, 1962, **41**, 1989—1991).—The liver of poults receiving a vitamin B_{12}-deficient diet contained about 40% of the amount of vitamin present in livers of birds receiving sufficient vitamin B_{12}. The methionine-synthesising activity of the livers from the vitamin-supplemented poults was greater than that of the livers from the deficient poults. A. H. CORNFIELD.

Effect of sex, feathering, rate of growth and acetates on the chick's need for glycine. H. N. Waterhouse and H. M. Scott (*Poultry Sci.*, 1962, **41**, 1957—1960).—Female chicks carrying the sex-linked gene for rapid feathering did not require a higher level of dietary glycine than did slow-feathering males. The chick's glycine requirement was not affected by inherent growth rate. Addition of NaOAc or NH_4OAc in amount equiv. to 3% glycine to a glycine-deficient diet did not improve chick growth. Mortality increased when the NaOAc level of the diet was $\leqslant 2\%$. A. H. CORNFIELD.

Effects of unnatural day lengths upon maturation and egg production of the Japanese quail, *Coturnix coturnix japonica*. H. Abplanalp, A. E. Woodard and W. O. Wilson (*Poultry Sci.*, 1962, **41**, 1963—1968).—Japanese quail were exposed to artificial light cycles of 14—44 h. in total length, but providing in all cases a light period twice as long as the alternating darkness. Short cycles of 16 and 18 h. retarded sexual maturation in both males and females and tended to interfere with continuous laying patterns in some hens. The retardation of normal gonad function is probably the result of interference between the imposed short-day cycles and an internal cycle of about 24 h. on which the birds' reproductive physiology functions. A. H. CORNFIELD.

Effect of the hypocholesteremic agent SC-11952 on the laying hen. E. L. Nichols and S. L. Balloun (*Poultry Sci.*, 1962, **41**, 1982—1984).—Addition of SC-11952 (0·024—0·060 g./lb. of feed) to the diet of laying hens slightly increased body wt. after 14 days, but reduced egg production to a fair extent and had no effect on yolk-cholesterol level. A. H. CORNFIELD.

Toxicity of common and hairy vetch seed for poults and chicks. J. A. Harper and G. H. Arscott (*Poultry Sci.*, 1962, **41**, 1968—1974).—The seed of common vetch, *Vicia sativa*, L. var. Willamette, caused high mortality when fed at 30—40% in poult diets and 20—40% in chick diets. Autoclaving the seed (8 h. at 15 p.s.i.) prevented mortality, but wt. gains with 2—40% autoclaved seed in the diet were not as satisfactory as with maize. Soaking the seed in water for 24 h. did not detoxify it. The seed of hairy vetch, *Vicia villosa* var. *glabrescens*, Koch, was not toxic to poults and chicks, but wt. gains were reduced in comparison with a maize diet. Autoclaving hairy vetch seed increased its growth-promoting effect. A. H. CORNFIELD.

Selenium content of chick tissues as affected by arsenic. C. W. Carlson, P. L. Guss and O. E. Olsen (*Poultry Sci.*, 1962, **41**, 1987—1989).—Addition of Se (10 p.p.m. as Na_2SeO_3) to the diet of chicks reduced wt. gains and increased liver Se. Arsanilic acid 0·01% in the feed reduced to some extent the growth inhibition due to Se and increased liver Se levels. As (15 p.p.m. as $NaAsO_2$) did not reduce wt. gains, did not prevent wt.-gain reductions due to 10 p.p.m. Se and increased liver-Se levels to a greater extent than did 0·01% of arsanilic acid. A. H. CORNFIELD.

Diseases of animals. N. N. Hole (*J. R. agric. Soc.*, 1962, **123**, 181—194).—Short summaries are presented of recent research on the nature, development and prevention of some diseases and disorders in livestock, notably rabies, groundnut poisoning, hypomagnesaemia, swine fever and fowl pest. A. G. POLLARD.

Livestock parasite control with broad-spectrum systemics. J. L. Lancaster, jun. (*Ark. Farm Res.*, 1962, **11**, No. 3, 7).—Successful control of horn flies and grubs by spraying with Co-Ral or ruelene (**I**) at concn. 0·25% in summer and 0·1% in winter is recorded. When poured over the backs of cattle **I** (dil. 1 : 4) almost completely eliminated lice. A. G. POLLARD.

Animal foodstuffs and compositions containing nitroiminazoles. Société des Usines Chimiques Rhône-Poulenc (B.P. 888,675, 6.7.60. U.S., 8.7.59).—Histomoniasis in animals is treated or prevented by adding to the feed or drinking water 0·001—0·1 wt.-% of a 4- or 5-nitroimidazole optionally substituted in the 1- and/or 2-position by alkyl (of total C $\geqslant 4$), e.g., 5-nitro-1,2-dimethylimidazole. F. R. BASFORD.

Salt of the levulinic acid derivative of cyanoacetic acid hydrazide. Farbenfabriken Bayer A.-G. (B.P. 888,679, 27.10.60. Ger., 28.10.59).—The piperazine salt of the hydrazide obtained by interaction of levulinic acid with cyanoacetic acid hydrazide ($CN{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}NH_2$) is suitable for use in the treatment of lungworm infection in cattle and sheep. F. R. BASFORD.

Ensilage of green fodder. Dr. Plate G.m.b.H. (Inventor: F. Raabe) (B.P. 888,656, 9.4.58).—The ensilage of green fodder is effected with a formate–nitrite mixture as an ensilage salt, the spreadability of the ensilage salt being ensured by addition of ($\sim$1 wt.-%) of a metal (Al) stearate. J. M. JACOBS.

Animal growth stimulation compositions. Olin Mathieson Chemical Corp. (B.P. 887,301, 20.6.58. U.S., 22.7.57).—A feedstuff for stimulating growth of animals comprises an edible carrier containing a broad-spectrum antibiotic and (as synergist) nystatin or amphotericin (3—6000 p.p.m.). F. R. BASFORD.

Device for use in determining the moisture content [of hay and like materials]. British Electrical & Allied Industries Research Ass. (Inventors: P. G. Finn-Kelcey and D. C. Perry) (B.P. 880,395, 9.2.59).—The device is based on the fact that the moisture content of a fibrous material is related to the amount of compression suffered by the sample when subjected to a predetermined pressure. In the device described a weighed amount of sample is compressed by a piston with a definite pressure and the length of the sample then measured. The apparatus is calibrated with material of known moisture content determined by drying. J. M. JACOBS.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Microbiological methods for the examination of cereals. J. Janicki, St. Stawicki, H. Maruszewska, H. Mozelewska and H. Pazdro (*Getreide u. Mehl*, 1962, **12**, 121—127).—The literature on microbiological examination of cereals is reviewed and the results of extensive trials of the effects of differences in sampling method, sample treatment, media recipes, and temp. and duration of culture on the enumeration of micro-organisms are reported and discussed. Suggested standardised procedures for the enumeration of total viable bacteria, bacterial spores and fungal spores are described. (20 references.) E. C. APLING.

Analysis of vitamins in cereals and cereals products. J. Janicki and E. Kamiński (*Roczn. Technol. Chem. Żywności*, 1962, **9**, 5—16).—Samples of the same wheat grain and wheat flour were sent (by Int. Ass. Cereal Chem.) to 21 laboratories in 14 countries. The results of determinations of vitamins B_1, B_2 and PP by chemical methods, by a uniform Swiss procedure, each laboratory's own procedure and by microbiological methods were compiled, tabulated and discussed. With vitamin B_1 chemical methods gave results varying from 2·92 to 6·9 μg./g. in the grain and from 0·61 to 2·33 μg./g. in the flour. The discrepancies were greater (22·6 and 38·8% respectively) with the Swiss procedure and smaller (20·8 and 26·6% respectively) with each laboratory's own procedure. More recent results have shown that 10—17% losses in vitamin B_1 occur when standard solutions are heated in the presence of glucose, maltose, sucrose or Fe^{2+}. With vitamin B_2 results by the fluorimetric method varied from 0·49 to 1·70 and from 0·17 to 1·00 μg./g. in the grain and flour respectively. The microbiological method gave results close to these, but the lumiflavine method results varied from 0·70 to 3·6 and from 0·16 to 1·08 μg./g. respectively. No decision was made on which method is preferable and no standard procedure for the hydrolysis and the liberation of vitamin B_2 bound with the proteins and metals was available. In the case of vitamin PP both the chemical and microbiological methods were in good agreement, but the Swiss procedure gave less consistent results.

In vitamin PP determinations the use of alkaline medium for hydrolysis was recommended. (15 references.)
A. L. GROCHOWSKI.

Absence of thiamine phosphates from cereals. N. P. Sen and A. D. Robinson (*Canad. J. Biochem. Physiol.*, 1963, **41**, 97—100).—A method is described for the separation and determination of thiamine and its mono- and diphosphates in cereals by extraction with $HClO_4$, separation on a column of Decalso, elution with strong aq. KCl and anion-exchange chromatography with Dowex-1. The thiochrome method with and without prior enzymic digestion was used for determination. Two varieties of each of stored wheat, barley and oats were analysed. Each contained free and bound thiamine (**I**). The analyses indicate that **I** is not in the form of phosphates. (20 references.) E. M. J.

Quick method [of determination] for riboflavin in a high-potency cereal product. R. H. Anderson and D. F. Moran (*Cereal Chem.*, 1962, **39**, 463—468).—The method, suitable for control purposes, depends on extraction of riboflavin from high-potency cereal foods with AcOH (10%). Addition of methanol (2 vol.) to the extract yields a clear, colourless filtrate suitable for fluorimetric analysis.
E. C. APLING.

Thermal properties of grain. E. A. Kazarian (*Dissert. Abstr.*, 1962, **23**, 1638).—The objective of this study was to determine the sp. heat, thermal conductivity and thermal diffusivity of soft white wheat and yellow dent maize and to relate these properties on the basis of moisture content and temp. The sp. heat and thermal conductivity of both wheat and maize increased linearly with moisture content. The thermal diffusivity of both grains decreased with increasing moisture content. F. C. SUTTON.

Rationalisation of protein determinations. W. Ludewigs (*Getreide u. Mehl*, 1962, **12**, 140—144).—A brief historical review of methods for determination of total N, with special reference to recent developments in rapid and automatic techniques applicable to cereals and cereal products. E. C. APLING.

Dependence of the properties of rye flour protein on extraction rate. V. Podrazky, J. Simová, Z. Vesely and F. Vones (*Getreide u. Mehl*, 1962, **12**, 127—129).—Results of determinations of total protein, globulin, prolamine, glutelin and water-sol. protein are reported for wholemeal and a series of rye flours milled to different extraction rates. No significant variation in gluten quality was detected, but total protein content and the relative contents of globulin and prolamine increased with increasing extraction rate. (10 references.) E. C. APLING.

Advances in the chemistry and evaluation of wheat. H. Neukom (*Schweiz. landw. Forsch.*, 1962, **1**, 281—297).—The chemical composition of wheat endosperm, germ and bran is discussed. The chemistry of the most important components of flour: starch, protein, enzymes and vitamins, is extensively reviewed. Storage and bulk handling of flour are discussed briefly. (49 references.)
J. V. RUSSO.

Using a density gradient column to determine wheat density. W. R. Peters and R. Katz (*Cereal Chem.*, 1962, **39**, 487—494).—The construction and use of a density gradient column, filled with mixtures of CCl_4 and cyclohexane, and standardised with glass beads of known sp. gr. is described. The density of individual kernels can be measured accurately to three decimal places. Relationships between *d* and moisture, protein and ash contents of the kernels, and application of the method to the detection of insect-infested grain, are briefly reported. E. C. APLING.

Variations in the amino-acid composition of Australian wheats and flours. D. H. Simmonds (*Cereal Chem.*, 1962, **39**, 445—455).—The amino-acid composition of six samples of Australian wheats and the flours milled from them on a Buhler laboratory mill was determined by the ion-exchange chromatographic method of Moore, Spackman and Stein. Fractionation of the proteins by differential extraction showed the pyrophosphate-sol. proteins (albumin–globulin group) to be richer in arginine and lysine and the gluten group proteins to be richer in glutamic acid. (26 references.) E. C. APLING.

Glutamic acid decarboxylase activity as a measure of damage in artificially dried and stored maize. G. M. Bautista and Pekka Linko (*Cereal Chem.*, 1962, **39**, 455—459).—A rapid method for the estimation of storage deterioration of artificially dried maize is based on determination of glutamic acid decarboxylase activity (GADA), using the Blish and Sandstedt pressureometer. Evolution of CO_2 in 30 min. at 30° from 15 ml. of 0·1M-glutamic acid in 0·067M-phosphate buffer at pH 5·8, by 30 g. of ground sample, measured in the pressureometer in terms of mm. of ethyl lactate is taken as a measure of GADA activity. GADA activity shows high correlation with viability, and all maize samples of better than 75% germination showed GADA activities equivalent to >200 mm. ethyl lactate. (18 references.) E. C. APLING.

Volume increase of kernels of maize and sorghum accompanying absorption of liquid water. Liang-Tseng Fan, Pu-shan Chu and J. A. Shellenberger (*Biotechnol. Bioengng*, 1962, **4**, 311—322).—The relationship between vol. increase and wt. increase of maize kernels during steeping is almost linear. The rate of vol. increase may be expressed as a diffusion equation and the coeff. of vol. increase obeyed the Arrhenius equation. W. ELSTOW.

Modified Alveograph method for hard winter wheat flour. M. D. Shogren, K. F. Finney, L. C. Bolte and R. C. Hoseney (*Agron. J.*, 1963, **55**, 19—21).—Differentiation, ranking and replicability were improved by modifying the original Alveograph method for testing the quality of hard winter wheat flour. A. H. CORNFIELD.

Correlations of certain properties of the alveogram with important hard winter wheat quality characteristics. M. D. Shogren, K. F. Finney, R. C. Hoseney and L. C. Bolte (*Agron. J.*, 1963, **55**, 21—24).—Alveogram area was not as good as alveogram length as a means of predicting protein content and loaf vol. Alveogram length (with protein held constant) and loaf vol. (corrected for protein) in general increased with mixing time within a variety. Alveogram length by the modified method was useful in predicting loaf vol. of hard winter wheat flour when mixing or oxidation requirement was taken into account and in measuring the deleterious effects imposed on hard winter wheat by γ-irradiation and excessively high drying temp.
A. H. CORNFIELD.

Methodical investigations of the sedimentation test [Zeleny test]. W. Seibel (*Getreide u. Mehl*, 1962, **12**, 133—140).—Extensive studies on the variability of sedimentation value with model mill employed, extraction rate, granularity, ash and protein contents, and temp., are reported. Collaborative tests showed good reproducibility between laboratories using fully standardised procedures. There was general agreement between sedimentation values obtained on flour from Tag-Heppenstall and Miag model mills, but somewhat higher values resulted on flour from the Brabender Quadrumat model mill. Current controversies on the utility and reproducibility of the test are briefly reviewed. (25 references.) E. C. APLING.

Tainting of flour with nitric oxide and the excessive sensitivity of the Griess–Ilosvay reagents. J. Buré (*Getreide u. Mehl*, 1962, **12**, 129—130).—Numerous possible sources of non-significant contamination of flour with minute traces of NO, detectable by Griess–Ilosvay reagents, are enumerated. The extreme sensitivity of the test severely limits its utility for the detection of flour treatment with NO_2 improver. Proof of such treatment is most simply and surely obtained by a full inspection of the mill. E. C. APLING.

Flour as a factor in bread firming. J. G. Ponte, jun., S. T. Titcomb and R. H. Cotton (*Cereal Chem.*, 1962, **39**, 437—444).—Crumb firmness and firming rates on staling (measured with the Baker compressimeter) showed significant differences among ten flours of similar analytical and test baking values. Firming rates did not correlate with protein, ash, maltose figure, amylograph value or starch damage, but there was significant correlation with proof time. Loaf centres were firmer than loaf ends and these intra-loaf differences correlated with specific vol. variations within the loaves. (11 references.) E. C. APLING.

Note on a rapid method for the estimation of damaged starch in soft wheat flours. J. R. Donelson and W. T. Yamazaki (*Cereal Chem.*, 1962, **39**, 460—462).—The method is basically the standard Blish and Sandstedt ferricyanide procedure for the determination of flour maltose figure, with the digestion conditions suitably modified. The sample (1·0 g.) is digested for 15 min. at 30° with the addition of 0·10 g. of Rhozyme 33 (a fungal diastatic enzyme produced from *Aspergillus oryzae*, and standardised to contain 5000 SKB units of α-amylase activity per g.). The maltose obtained is equated to damaged starch by multiplying by the empirical factor 1·64. (Gelatinised starch yields 61 ± 0·43% maltose under these conditions.) The results obtained on soft wheat flours agreed well with those of the Sandstedt and Mattern procedure (*Cereal Chem.*, 1960, **37**, 379). E. C. APLING.

Oxidation of wheat starch with chlorine. Norito Uchino and R. L. Whistler (*Cereal Chem.*, 1962, **39**, 477—482).—Oxidation of dry or semi-dry wheat starch by Cl_2 is slow; the rate increases with rise in temp. and moisture content of the starch, and is more rapid in light than in darkness. Hydrolysis of the oxidised starch yielded D-gluconic acid (equivalent to about 42% of the Cl consumed) and small amounts of D-glucuronic acid, indicating that the main attack is on carbon atom C 1. The extent of oxidation suggests that some depolymerisation of starch occurs. E. C. APLING.

Enzymic hydrolysis of wheat starch. I. Rapid method for colorimetrically estimating amylase activity. S. Rogols and R. L. High (*Stärke*, 1963, **15**, 1—4).—Wheat starch was studied in terms of products obtained by (commercial) enzyme hydrolysis; two

variables, temp. and pH, were examined. The method is based on the ability of dextrin-like polysaccharides to combine with I and extrapolation of obtained data to a standard dextrin–I curve. As conditions become optimal for enzyme activity the total activity of the enzyme, in terms of the reaction products, is constant within defined environmental conditions; the composition of the reaction products changes markedly. E. M. J.

Action of some α-amylases on starch granules. G. J. Walker and P. M. Hope (*Biochem. J.*, 1963, **86**, 452—462).—Human salivary α-amylase rapidly degrades maize-starch granules. The degrading activity relative to hydrolysis of dissolved starch varies with pH and temp. Adsorption of the enzyme on the surface of the granules increases at low temp. and high pH values. Pig pancreatic α-amylase has a similar hydrolytic action on raw starch and behaves similarly on adsorption. The α-amylase from *Bacillus subtilis* hydrolyses starch grains slowly and is adsorbed on the granules at 0° only, and not at 35°. *Aspergillus oryzae* α-amylase and sweet potato β-amylase do not degrade maize-starch granules, and are not adsorbed on the granules. None of these amylases degrades potato-starch granules. J. N. Ashley.

Ageing and maturing of baker's yeast. R. Kubíček and Z. Potěšil (*Kvasný průmysl*, 1963, **9**, 57—61).—The reasons for differences between the actual lifetime of the product and the durability determined by laboratory tests in liquefying yeast at 35° have been studied. The behaviour of yeast depends largely on the stage of its maturity, i.e., on its enzymic activity. The maturing process continues during the storage period. Therefore, durability tests based on liquefaction are not reliable enough, their results being affected by the factors of ageing and maturing. J. S. B.

Flocculation of yeast. A. Ginterová (*Kvasný průmysl*, 1963, **9**, 11—14).—The troubles caused by yeast flocculation in baker's yeast production plants are described and the causes discussed. The problems of flocculation are extremely important, and should be duly understood prior to changing the existing batch operation to a continuous process. The knowledge of the nature of processes involved is at present not clear enough for explaining the causes of flocculation and to suggest effective countermeasures. (18 references.) S. J. B.

Determining baker's yeast agglutination by the sedimentation method. A. Ginterová, L. Mitterhauszerová and O. Janotková (*Prům. potravin*, 1963, **14**, 97—98).—A method has been developed for determining the sedimentation of baker's yeast. Sedimentation data permit the evaluation of the agglutination properties of the samples examined. The method described is a modification of that of Burns, Helm *et al.*, and consists in the use of a phosphate buffer of pH 4·5. Two types of agglutination could be distinguished, one characterised by flakes, the other by grains. J. S. B.

Function of trehalose in baker's yeast (*Saccharomyces cerevisiae*). A. Panek (*Arch. Biochem. Biophys.*, 1963, **100**, 422—425).—Trehalose (**I**) and glycogen, which are storage carbohydrates of this yeast cell, are found in non-proliferating cells in a ratio of 5 : 1. During aerobic starvation **I** is not utilised by the cells but during the initial lag-phase of the growth curves this reserve is rapidly mobilised and consumed to a 90% extent, CO_2 being mostly formed in the degradation. The liberated energy is possibly used for cell division. C. V.

Small-scale pressurised dough developer for the laboratory. T. A. Mitchell (*J. Sci. Fd Agric.*, 1963, **14**, 239—244).—A predetermined pressure can be maintained within the developer independently of the dough consistency or the throughput. The developer is similar in principle to the Do-Maker but has a capacity of only 575 ml. The pump is basically a piston pump. Compressed air admitted to one side of the piston applies pressure to the dough on the other side. The pressure within the developer is controlled by that of the compressed air supply, the work input by the development time, and the dough temp. by that of water circulated through a jacket on the developer. All these conditions can be varied independently; also the unit may be constructed from common materials with a min. of engineering equipment. Pieces (400 g.) baked at 480°F produced loaves of good vol. with fine texture. E. M. J.

Disulphide–sulphydryl exchange in dough. C. A. M. Mauritzen and P. Stewart (*Nature, Lond.*, 1963, **197**, 48—49).—The components of dough after mixing were separated by preparative ultracentrifugation and the incorporation of ^{35}S-cysteine and ^{14}C-leucine into the proteins examined. Only 4% of the leucine became bound to non-dialysable components of dough but with cysteine 11% of the isotope was bound to sol. proteins of the dough liquor and 24% to the gluten. S. A. Brooks.

Effects of iodate, *N*-ethylmaleimide [NEMI] and oxygen on the mixing tolerance of doughs. P. Meredith and W. Bushuk (*Cereal Chem.*, 1962, **39**, 411—426).—The effects of the two named sulphydryl reagents on flour/salt/water doughs mixed in N_2, O_2 and air were studied by the Farinograph and Extensograph and by estimation of the acid-insol. fraction of washed gluten (cf. P. Meredith, *N.Z. J. Sci.*, 1961, **4**, 66—77). Both reagents caused dough breakdown in doughs mixed under N_2 and at lower levels in doughs mixed in air or O_2, although NEMI was apparently more effective than iodate on an equivalent basis. NEMI also produced additional breakdown in iodate-treated doughs, but iodate was without effect on NEMI-treated doughs. The results are discussed in relation to current theories of the rôle of —SH groups in the chemical and physical changes occurring in doughs. (12 references.) E. C. Apling.

Investigation of possibilities for improving the quality and taste of wheaten and wheat–rye-mixture bread. A. Schultz and H. Stephan (*Brot u. Gebäck*, 1962, **16**, 203—209).—Studies of the crumb structure and plasticity and the aroma and taste of wheaten bread prepared from variously-soured doughs are reported. Improvements in quality and taste resulted from souring with small additions of organic acids (particularly citric and acetic acids) and from souring produced with pure cultures of lactic or propionic acid-producing bacteria. A commercial lactic acid bacteria/yeast mixture produced well-flavoured bread from both wheat and wheat/rye doughs. E. C. Apling.

Production of bread after radioactive contamination of raw materials. H. D. Ocker (*Brot u. Gebäck*, 1962, **16**, 212—216).—The probable effects of large-scale accidental or warlike radioactive contamination of foodstuffs, methods of prevention and of decontamination are outlined and discussed with special reference to bread supply. Although large-scale decontamination of many foodstuffs is impracticable, good milling techniques produce flour substantially free from ^{90}Sr and ^{137}Cs from contaminated wheat. The problems involved in ensuring the final production of uncontaminated bread are enumerated and discussed. E. C. Apling.

Origin and fate of certain carbonyl compounds in white bread. Yu-Yen Linko, J. A. Johnson and B. S. Miller (*Cereal Chem.*, 1962, **39**, 468—476).—A study of the effects of bakery techniques and storage conditions on the development and retention of carbonyl compounds in bread is reported. Additions of sucrose to the dough resulted in increased amounts of hydroxymethylfurfural (and furfural) in the crust, but not in the crumb, and 'total carbonyls' generally increased with increasing crust colour. Additions of leucine and xylose gave more isovaleraldehyde (**I**) in the crust, but additions of **I** to dough were largely lost in fermentation and baking. 'Total carbonyls' in the crumb increased on storage up to 3 days and then fell rapidly; in the crust there was little change at first, followed by a rapid fall after 5 days of storage. (21 references.) E. C. Apling.

Changes in the moisture content of different regions of the crumb of bread during storage of uncut loaves. E. Drews (*Brot u. Gebäck*, 1962, **16**, 221—229).—Studies on the moisture distribution in the crumb of wheat/rye mixture loaves baked by different procedures, and of the changes occurring during storage for 14 days of both wrapped and unwrapped loaves are reported in detail and discussed. One day after baking different regions of the crumb have closely similar moisture contents, but differentiation increases with time of storage. Large moisture gradients arise in wrapped bread, and the extent of the changes in moisture distribution also varies with the original overall moisture content of the loaf, its shape and crust character, and on conditions of cooling and storage. (22 references.) E. C. Apling.

Influence of egg quality on the structure and colour of the crumb in fancy baked goods. A. Rotsch and E. Tehsmer (*Brot u. Gebäck*, 1962, **16**, 201—203).—A brief discussion of the importance of egg yolk colour in fancy baking, and the use of frozen egg yolks for the improvement of crumb colour, with special reference to the production of panettoni. E. C. Apling.

Cold-swelling starch flour. A. Oetker Nahrmittelfabrik G.m.b.H. (B.P. 888,687, 14.5.58. Ger., 14.5.57).—A cold-swelling starch flour, suitable for use in the prep. of puddings without cooking, is produced by working up a suspension of (potato or maize) starch flour into a paste, then feeding the paste in an aerated condition to a roller dryer, e.g., working at 4 r.p.m. and heated with steam at 155°/4·5 atm. F. R. Basford.

Potato-base foodstuffs. W. Stewart & Arnold Ltd. (Inventors: W. J. S. Peschardt and S. R. Hume) (B.P. 888,689, 28.10.58).—A potato-base foodstuff (which on frying in oil will expand and puff up like popcorn) is prepared by mixing potato flour (dried to 4—5% of moisture and sieved to $<$100 in. mesh) with 2 pt. of potato starch; adding water; subjecting the dough (preferably after forming into a continuous rod, then cutting into short lengths) to boiling water,

so as to gelatinise the starch and cause swelling of the dough ; then drying to rubber-like consistency. If desired, the boiling water may also contain a small amount of Na alginate and Na_3PO_4.
F. R. BASFORD.

Sugars and confectionery

Decolorising ion exchangers. XIX. Comparison of new types of decolorising ion exchangers. J. Štamberg and Z. Vašíček (*Listy cukrovar.*, 1963, **79,** 33—38).—In comparison with older types (polycondensates) the new types of decolorising ion-exchangers (polyamines and polystyrenes) are characterised by higher sorbent capacity towards the colouring matter of molasses, and above all they are less susceptible to exhaustion in repeated cycles. A markedly low decrease of the decolorising capacity under conditions experimentally applied was shown by the new types of Amberlite XE-168 and Decolorex A 5. The decrease of decolorising capacity during repeated cycles is caused by destruction of the exchanging groups, as by incomplete regeneration and gradual clogging by the colouring matter. The effect of remedial regeneration is of short duration. Therefore it is preferable to use resins characterised by the min. tendency to clogging during repeated cycles. J. S. B.

Determination of sucrose and invert sugar by direct polarisation. B. Tichá and M. Friml (*Listy cukrovar.*, 1963, **79,** 57—62).—The influence of borax on the optical activity of glucose, fructose and sucrose and mixtures thereof was studied in order to simplify polarimetric methods for determining sucrose in the presence of invert sugar. The optical activity of said sugars is lowered by addition of tetraborate, the influence of which with various ratios of components was examined, and the application range of direct polarimetric determination established. On the basis of this knowledge and with regard to the influence of the usual clarifying agents, which is additive, the following conditions for direct polarimetric determination of sucrose have been derived: (*a*) 0·5 g. $Na_2B_4O_7,10H_2O$ in 100 ml. N-sucrose, after clarification with basic Pb acetate, the optical activity of invert sugar is eliminated up to 2·3% in a sample; using 0·5N-sucrose the application limit is shifted up to 4·6% of invert sugar. (*b*) 0·5 g. $Na_2B_4O_7,10H_2O$ in 100 ml. N-sucrose, after clarification with basic Pb nitrate (Herles' reagent), the activity of invert sugar is eliminated up to 6% ; using 0·5N-sucrose up to 12% content in the sample. J. S. B.

Microbiological standards for sugar. M. P. Scarr (*J. Sci. Fd Agric.*, 1963, **14,** 220—223).—Microbiological standards for white sugar imply freedom from pathogens, the lowest practical no. of spoilage organisms and a guarantee of hygienic production. The types and no. of organisms present in dry and (especially) liquid sugar are discussed with reference to the soft drinks industry, production of commercial sugar syrup (**I**) and sugar for canning purposes. With **I**, in addition to preliminary hygienic prep. of the storage tanks, the air over the surface of the liquor must be filtered and u.v. lamps may be used in the tanks to sterilise condensation. E. M. J.

Thin-layer chromatography of malto-oligosaccharides. C. E. Weill and P. Hanke (*Analyt. Chem.*, 1962, **34,** 1736—1737).—Kieselguhr G with solvent mixtures of butanol–pyridine–water, butanol–lutidine–water and butanol–ethanol–water, were effective in separating malto-oligosaccharides of up to 10 glucose units.
G. P. COOK.

Discoloration of maize syrup by light. B. L. Scallet (*Stärke*, 1963, **15,** 4—6).—Development of coloured compounds in maize syrup during storage is discussed, such reactions being catalysed by heat, acids, alkalis, metallic ions and oxidising and reducing agents. An unusual brownish colour which develops in presence of traces of Cu contamination under the influence of sunlight is described.
E. M. J.

Non-enzymic browning reactions : consideration of sugar stability. H. S. Burton and D. J. McWeeny (*Nature, Lond.*, 1963, **197,** 266—268).—Various findings connecting rate of browning with the configuration of the sugar involved are reviewed. One factor in the speed of development of initial chromophores appears to be the actual conformational stability of the sugar. (16 references.)
S. A. BROOKS.

Citric acid products. C. Pfizer & Co. Inc. (B.P. 887,973, 30.5.58. U.S., 2.8.57).—A surface-hydrated citric acid, suitable for confectionery use, is produced by adding pure water (3—6%) to fine, granular citric acid (U.S.P. grade) of 60—100 in. mesh (U.S. Standard sieve size) at 0—35° with stirring. F. R. BASFORD.

Fermentation and Alcoholic Beverages

Variations in grape juice in course of pressing. J. Carles, A. Alquier-Bouffard and J. Magny (*C. R. Acad. Agric. Fr.*, 1962, **48,** 773—780).—Samples taken periodically from non-continuous presses (Vaslin and Pneumabil) show a continuous increase in the content of N compounds, the final content being > 50% > the initial content. The Fe and Mn decrease very rapidly at first, and increase moderately in the final pressings. The Cu shows the highest values during the middle period. The other inorg. elements remain moderately constant or show moderate decreases followed by increases (especially in the P) during the final pressings.
P. S. ARUP.

Aromatic substances in fermentation products. J. Hrdlička and J. Kubíček (*Kvasný průmysl.*, 1963, **9,** 35—38).—Results of analyses of four sorts of white and two sorts of red wine are reported, explaining to a certain degree the factors determining the proportion of amino-N to total N. By aid of paper chromatography 16 components were isolated and identified. For securing correct identification various methods were applied, e.g., comparison with standard samples, comparison of R_F values, various development procedures employing different developers, etc. The presence of aliphatic amines of the C_1—C_5 range, diamines and polyamides has been ascertained. (19 references.) J. S. B.

Acids and colouring matter present in wines. I. Mareca Cortés and C. Diez de Bethencourt (*Chim. anal.*, 1962, **44,** 527—532).—Two methods for the determination of acids and colouring matter in wines, as a measure of their degree of ageing, are described. The chromatography of artificial mixtures of tartaric, malic, citric, lactic and succinic acids in alcoholic solution together with bromophenol blue was studied as a model system. A photometric method was used to study the changes in light absorption (at 440 and 524 mμ) of wines with increasing age. P. D. PARR-RICHARD.

Detection of wine in spirits. II. Methods of examination for brandy and liqueurs, including egg liqueurs. K. Neumann (*Dtsch. Lebensmitt-Rdsch.*, 1962, **58,** 360—363).—Modifications of the general methods (*Dtsch. Lebensmitt Rdsch.*, 1962, **58,** 107) for the examination of distilled liqueurs and emulsified liqueurs are briefly described. In the particular case of egg-cognac, the beverage is diluted with water (3 vol.), shaken for 15 min. and centrifuged to remove lipoids. The clear aq. liquid is then examined chromatographically for glycerol. The method will detect the addition of 2·5% of wine in egg liqueur. (11 references.) E. C. APLING.

Extraction of higher fatty acid esters of alcoholic beverages and their analysis by gas–liquid chromatography. M. J. De Vries (*S. Afr. J. agric. Sci.*, 1962, **5,** 395—400).—A 300—500 ml. sample of the beverage is extracted with a 2 : 1 ether/pentane mixture and the extract concentrated down to 1 ml. This is analysed on a 2 m. column containing 20% polyethylene glycol succinate as stationary phase. Good separation is achieved at a column temp. of 188° and a gas flow of 68 ml. He/min. For accurate quant. analysis a known amount of ethyl nonoate or ethyl hendecanoate is added before extraction. Both these esters are exceptionally well suited for use as internal standards. Recoveries from 96—102% were obtained from model solutions. (10 references.) S. G. AYERST.

Effect of the ultrasonic field on the sprouting ability and diastatic power of brewery barley. S. Bachman and M. Kenner (*Roczn. Technol. Chem. Żywnosci*, 1962, **9,** 113—121).—Good quality brewery barley, either air-dried or soaked in water for 12 or 24 h., was treated for 2·5, 5·0, 7·5 or 10 min. in an ultrasonic field of approx. 15 W per sq. cm. of 0·5, 0·6, 1·0 and 2·0 MHz frequency. Best sprouting stimulating effect, whereby the no. of sprouting seeds was increased from 90% in the control untreated batch to 95—97% in the treated batch, was obtained in barley subjected to the frequencies of 0·5 and 0·6 MHz for 5 and 7·5 min., and in seeds soaked for 12 h. the same frequencies over 2·5 and 5 min. The above conditions also speeded the sprouting process. All other tested conditions gave poorer results, the longer exposure times, particularly for seeds previously soaked, being more damaging than the higher frequencies used. The ultrasonic-treated barley, particularly the previously soaked batches, showed optimum diastatic power and max. amount of the reducing sugars between the fourth and the sixth day of malting. The 2·5—7·5 min. exposures resulted in hastening the sprouting by 2—3 days and increasing the diastatic power between the fourth and sixth day of malting. Correct ultrasonic treatment therefore can cut malting time by 25%. The literature on the subject is reviewed and results are correlated with those of Specht *et al.* (*Brauwelt*, 1953, **39A,** 508) and Busnell and Obolensky (*Ultraschall*, 1955, **8,** 8). (18 references.) A. L. GROCHOWSKI.

Hartong value and its application in connexion with brewing and malting. R. Scriban (*Brauwissenschaft*, 1963, **16,** 4—11).—The results of numerous analyses of French malts show close correlations between the Hartong value and the saccharification value (VZ) at 45° and between the VZ at 45° and the VZ at 20° (r = 0·95 and 0·87). The correlations between the VZ at 45° and the Kolbach value, the

diastatic value, and the solubility difference between fine and coarse grist (negative) are of a lower order. The equations connecting the Hartong value with the VZ at 45° for the seasons 1960 and 1961 show very small differences. The Hartong test should be carried out at pH 5·9 and at 20° as well as at 45°; the test at 45° is mainly influenced by the α-amylase content. Hartong values at 45° of 0—3·5 indicate poor solubility whilst values >5·5 indicate over-solubility. Good malts should show VZ (at 20°) values of 22—26% and VZ (at 45°) values of 30—36%. The relationships of the Hartong value with respect to various criteria are considered with reference to other published data. (12 references.) P. S. ARUP.

Determining the brittleness of malt and its uniformity. Z. Šauer, J. Voborský and T. Lejsek (*Kvasný prûmysl*, 1963, **9**, 3—9).—A commercial apparatus for testing grain kernels was adapted for determination of the brittleness of malt and of its resistance against crushing. The tester measures the force required to cut the grain kernel crosswise, reflecting the brittleness of the tested grain. The mean square deviation is taken as the criterion of uniformity, indicating the homogeneity of malt. The values obtained in large-scale series of tests confirm the suitability of the modified apparatus for malt analyses. The results stand in an established relation to the results obtained by comparing extracts from finely and roughly crushed malt. There is also a correlation to the Kolbach no. No definite relation to results of mechanical tests could be ascertained. (16 references.) J. S. B.

Paper-chromatographic determination of fermentable sugars in comparison with attenuation by yeasts. II. W. Kleber, P. Schmid and I. Seyfarth (*Brauwissenschaft*, 1963, **16**, 1—4).—The prep. of pure maltotriose (**I**) from wort is described. After exhaustive fermentation with *Saccharomyces uvarum*, membrane filtration and removal of the proteins, the wort is concentrated by evaporation; the (unfermentable) **I** is separated from the accompanying higher polymers of maltose by column chromatography on active C–Celite 535 (1 : 1) and fractional elution with water followed by water–ethanol with increasing concn. of EtOH. The previously described paper-chromatographic method (cf. *Anal. Abstr.*, 1961, **8**, 4837) is applied to solutions of **I** alone and in admixture with 2% of fructose, glucose or sucrose, or with 5% of maltose; the linear and coincident calibration graphs show consistent results for **I** and for the added sugars. Reproducibility data show deviations of 2·9—4·5% from the mean values. (13 references.) P. S. ARUP.

Clarification of beverages. Dow Chemical Co. (B.P. 887,796, 5.12.58. U.S., 9.12.57. U.S., 7.5.58).—A beverage (especially alcoholic malt beverage, e.g., beer, ale, porter) is clarified and stabilised against hazing by adding a 1-vinyl-2-oxo-oxazolidine (0·0001—1%) (to coagulate and precipitate haze-inducing ingredients), then removing the ppt. if desired. The preferred additive is a polymer or copolymer containing 60—80% of 1-vinyl-5-ethyl-2-oxo-oxazolidine, preferably introduced into the beverage at <4° (followed by warming to >4°). F. R. BASFORD.

Fruits, Vegetables, etc.

Inhibition of the evolution of ethylene and the ripening of fruit by ethylene oxide. M. Lieberman and L. W. Mapson (*Nature, Lond.*, 1962, **196**, 660—661).—When green tomato fruits were held for 16—22 h. in an atm. containing 0·75% ethylene oxide ripening was retarded and ethylene production inhibited. Retarded fruit ripened normally after the delay and flavour was not affected. Similar results were obtained with apples. Acetaldehyde also retarded ripening. S. A. BROOKS.

Firmness of canned apple slices as affected by maturity and steam-blanch temperature. R. S. Shallenberger, J. C. Moyer, R. L. LaBelle, W. B. Robinson and D. B. Hand (*Food Technol.*, 1963, **17**, No. 1, 102—104).—As harvest date was delayed, the fruit was less hard, but provided firmer canned slices. The firmness was associated with a higher ratio of alcohol-insol. solids (AIS) to total solids (TS) in the raw fruit. The extent of firming was a joint function of steam blanch temp. (**I**) and AIS/TS ratio (**II**). With a low **II** canned slice firmness first increased with higher **I**, then decreased. With higher **II** firmness increased exponentially as **I** were raised. E. M. J.

Chemical and colour changes in canned apple sauce. B. S. Luh and P. J. Kamber (*Food Technol.*, 1963, **17**, No. 1, 105—108).—Apple sauce made from Gravenstein apples and sucrose under commercial processing conditions was tested for storage stability at 32—98°F. Undesirable chemical and colour changes occurred at 86° and 98°F. Hydroxymethylfurfural formation and sucrose inversion (rapid at higher storage temp.) occurred. Canned apple sauce should be stored at ≯68°F. (15 references.) E. M. J.

Factors affecting quality of pies prepared from frozen bulk-pack red sour pitted cherries. D. G. Guadagni, J. Harris and K. M. Eremia (*Food Technol.*, 1963, **17**, No. 2, 103—106).—In the commercially packed frozen cherries stored at 0—30°F, flavour was more stable than colour. Flavour changes occurred in 1—2 weeks at 30°, 13—18 weeks at 20°, 27—35 weeks at 10° and 78—104 weeks at 0°F. In cherries packed in 30-lb. cans stored at 10—30°F, the surface layers (5—10% of the contents) were discoloured. When this proportion of brown cherries was mixed with the remaining can contents, the colour and flavour of the cherry pies was not significantly affected. Rapid freezing reduced the browning of the surface layers The cherries stored at 10—30°F became firmer. (11 references.) E. M. J.

Non-alcoholic beverages

Composition, production and assessment of so-called 'whole fruit' orange juices. E. Benk (*Riechstoffe u. Aromen*, 1963, **13**, 18—21).—Two types of these 'whole fruit' juices appear on the market, viz., a true whole fruit juice made by crushing the fruit (and containing only minor amount of peel, etc., constituents), and a juice obtained by adding ground-up peel with or without flavouring additives or β-carotene. Descriptions are given of the production, analytical composition, and properties of each type, and their use in making soft drinks. H. L. WHITEHEAD.

Preservation of fruit juices by chemical means. P. Dupaigne (*Fruits, Paris*, **17**, 547—556).—A short review of the available chemical means of preserving fruit juices. These preservatives are discussed under the following headings. Natural preservatives used in large doses, e.g., sugar and preservatives used in small doses. This latter group is divided into those used for temporary preservation, e.g., SO_2 and N_2, those which only have temporary action, e.g., diethyl pyrocarbonate, antibiotics and the stable antiseptics, e.g., benzoic acid. Points for and against the use of preservatives are discussed. A large bibliography is given. W. ELSTOW.

Foam-mat dried orange juice. I. Time–temperature drying studies. O. Bissett, J. H. Tatum, C. J. Wagner, jun., M. K. Veldhuis, R. P. Graham and A. I. Morgan, jun. (*Food Technol.*, 1963, **17**, No. 2, 92—95).—The technique of preparing powders from fruit juices (1961) is applied to the dehydration of orange juice. The effects of drying times and foam temp. (max. 160, 170 and 180°F during drying) were studied. Products dried at 160°F for 11·7—26·2 min. were of good flavour and moisture content varied from 4·55 to 2·71%; those dried at 170°F for 10·5—13·1 min. contained 3·99—3·37% moisture, flavour was good; those dried at 180°F for 8·8—13·1 min. contained 4·03—2·46% moisture and were of good flavour. (16 references.) E. M. J.

Tea, coffee, cocoa

Spectrophotometric detection of extracted cocoa fat in chocolate (additional note). W. Hennig (*Dtsch. Lebensmitt Rdsch.*, 1962, **58**, 363).—The incorporation of vanillin (30 g. per 100 kg.) in a milk chocolate containing 30% of fat results in a value of $E^{1\%}_{1\,cm.}$ 270 mμ = 0·7 by the method previously described (*ibid.*, 1962, **58**, 251). *E* values in excess of 0·65 indicate the presence of extracted cocoa fat only in the absence of vanillin, and the method must be considered only as a preliminary sorting test. E. C. APLING.

Chocolate. J. W. Greer Co. (B.P. 887,634, 11.11.60. U.S., 12.11.59).—Molten chocolate is cooled to give a product of improved texture by applying to the surface of the hot chocolate a heat-absorbing medium at a temp. lower than the subsurface temp. (of the chocolate) but high enough and for a period of time sufficient to induce crystallisation of the crystallisable ingredients and until after the point of inflexion in the time–temp. cooling curve of the subsurface caused by heat of crystallisation under the applied conditions (to 72—78°F); then decreasing the temp. of the medium, to increase the temp. differential between the medium and subsurface to a value greater than the initial differential between the two. F. R. BASFORD.

Milk, Dairy Products, Eggs

Physico-chemical properties of milk. XII. The renneting time of milk of different species. Balwant Rai Puri and Sat Parkash (*Indian J. Dairy Sci.*, 1962, **15**, 114—122).—Renneting times (**I**) of cow, buffalo and goat milk were determined at temp. ranging from 15 to 30° and at rennet concn. varying from 10 to 50 mg. per 100 ml. milk. **I** decreased with rise in temp. and of rennet concn. At low rennet concn. temp. rise had a greater effect on **I** of cow milk than on that of either buffalo or goat milk. Under similar conditions **I** of cow

milk was considerably greater than that of milk of the other two species, and this offers possibilities of providing a distinctive test for cow milk. Increase in acidity and addition of ionised Ca, Ba and Al lowered **I** of all milks while the replacement of ionic Ca by monovalent alkali cations raised the **I** considerably. (24 references.)
M. O'LEARY.

Composition of milk of dairy cows as influenced by feeding of L-thyroxine triturate. I. D. Desai and B. M. Patel (*Indian J. Dairy Sci.*, 1962, **15**, 105—113).—Administration of 1% L-thyroxine triturate at the rate of 8 g./day to Kankrej cows resulted in a slight, but not statistically significant, reduction in the lactose content of their milk. The chloride content increased. The lactose–chloride relationship was not affected. For this breed of cows, this relationship may be expressed by the formula : lactose % = 5·87 − 13·5 Cl %. The ash content of the milk was not affected by thyroxine feeding but there was a 100% increase in the I content and a significant decrease in the ascorbic acid content. (20 references.)
M. O'LEARY.

Influence of milk acidity and of its standardisation on the quality of Gruyère cheese. Č. Olšanský, H. Vychytová, F. Žák and Z. Chlup (*Prům. potravin*, 1963, **14**, 85—89).—To elucidate theoretical problems and to improve the technological process, the single phases of processing heated curd to Gruyère cheese were examined. High-quality cheese can be made only if the acidity of milk is 7·0—7·1°, the limit being 7·6°. Various methods of adjusting the acidity have been applied and results compared. The acidity can be adjusted as required either by introducing cream ferment prior to pasteurisation, or by adding lactic acid prior to curdling. Pre-ageing failed to give positive results. (15 references.) J. S. B.

Control of radioactive pollution of milk. G. Michon (*Le Lait*, 1961, May—June, 8 pp.; *Rapp. Cent. Et. nucl. Saclay*, 1962, No. 2118).—A general discussion of optimum procedures for sampling of milk during production and distribution and for subsequent analysis of samples (measurement of β- and γ-activity, determination of ^{90}Sr, ^{137}Cs, ^{131}I, ^{40}K and Ca). Data (whether at national, regional or local levels) should always permit reliable extrapolation to the entire output and to the prediction of future trends. W. J. BAKER.

Amido-black method for the determination of total protein in milk. K. F. Vogt (*S. Afr. J. agric. Sci.*, 1962, **5**, 433—437).—Two modifications, one using filtration (i) and one centrifuging (ii) of the Amido-black method were investigated and correlated with results obtained by the Kjeldahl method. Correlations of 0·912 for modification (i) and of 0·984 for modification (ii) were found. With modification (i) it was found also that absorbency values varied a good deal with different batches of the filter paper. Modification (ii) is of acceptable accuracy. S. G. AYERST.

Coagulation and electrophoretic behaviour of protein dispersion in milk on the addition of electrolytes. B. R. Puri and S. Parkash (*J. Indian chem. Soc.*, 1962, **39**, 605—611).—The coagulation of cow and buffalo milk by various salts was studied by determination of the lowest concn. at which turbidity occurred (flocculation value, FV). The FV was affected by both the anion and the cation. Zn had an outstandingly low FV and this is considered significant in view of the presence of Zn in rennin. Both cow and buffalo milk behaved similarly. Dilution with water increased the stability of milk to K and NH_4 oxalates but decreased it to $ZnSO_4$. Addition of other electrolytes to $ZnSO_4$ solutions had no effect up to 0·05 moles per l. but thereafter made the milk more stable. Negatively charged caseinate particles can take up ions carrying the same charge from solution. Addition of increasing amounts of NaCl, KCl, $CaCl_2$ and Na acetate resulted in increased electrophoretic mobility. NH_4 oxalate up to 0·005 mole per l. increased mobility but at 0·006 mole/l. (half of the ionic Ca is precipitated) the mobility decreased. With increasing concn. of $ZnSO_4$ an initial rise in mobility was followed by a fall. These results are considered to confirm the previous conclusion of the absorption of anions by casein particles in milk.
J. I. M. JONES.

Mechanised line for acid casein production. J. Čeřovský, J. Basař and V. Kněz (*Prům. potravin*, 1963, **14**, 64—66).—Newly developed machines for washing and pressing acid casein, suitable to be assembled to a continuous production line for acid casein, are described. A mechanised line thus arranged has a daily capacity of 300 hl. milk to be processed to thermophilic casein. On the condition that the temp. of the washing water was 50°, the average sugar content of the wet pressed casein amounted to 0·5%, and that of dry matter to 40—43%. J. S. B.

Changes in milk fat as a cause of certain defects in milk and milk products. E. Zollikofer (*Schweiz. landw. Forsch.*, 1962, **1**, 340—348).—Tallowy or fishy odours in milk can be traced to oxidation of unsaturated glycerides or of the protein membrane which surrounds the fat globules. Milk contains natural antioxidants but these are sometimes destroyed by heat treatments. Rancidity is due to hydrolytic fat splitting by the enzyme lipase. Milk from animals with ovarian cysts has a very high lipase activity and goes rancid very quickly. (15 references.) J. V. RUSSO.

Factors affecting the correlation between results obtained with the TeSa, Schain and Babcock butterfat tests. M. K. E. Ibrahim and W. F. Shipe (*J. Ass. off. agric. Chem., Wash.*, 1962, **45**, 908—915).—At 3—7% of fat the TeSa and Babcock methods gave similar results, but at 7—8% of fat the former method gave lower results. At 3—5% of fat the Schain test gave higher, at >5% of fat lower, results than the others. No breed effect was observed. Changing the boiling period (8—12 min.) did not appreciably affect the TeSa results. Addition of $HgCl_2$ or $Na_2Cr_2O_7$ as preservatives increased the TeSa but decreased the Babcock results. A. A. ELDRIDGE.

Proteolytic changes in butter. H. Jęsiak and J. Kisza (*Roczn. Technol. Chem. Żywnosci*, 1962, **9**, 123—138 ; cf. *Proc. XV Int. Dairy Congress, London*, 1959, **2**, 1036—1042).—Further studies on the protein decomposition and its effect on the quality and stability of butter are reported. Ten samples each of non-washed and washed butter were analysed immediately after prep., then after being kept 4 weeks at 20°, and four further samples after 6 weeks at 20°. The fat, water and dry non-fat content was tabulated for all fresh samples, and the acidity and pH of the plasma, the acidity of the fat present, the peroxide no., and the oxidation susceptibility of the plasma for all fresh samples and those stored for 4 weeks. Fresh unwashed butter had more intense aroma, slightly higher potential and active acidity, very high oxidation susceptibility, and was richer in the total, non-protein, protein, sol., peptone and amino-acid forms of N. On storage, the flavour and aroma of unwashed butter deteriorated to a greater extent and its sol. N content (10% of total N initially in all samples) increased more rapidly. In stored butter all forms of N, apart from the peptone N, increased progressively after 4 and 6 weeks. The amino-acid N, initially 34—41% of total sol. N in fresh butter, increased to 50 and 60% after 4 and 6 weeks respectively. Paper chromatography indicated the presence of 14 amino-acids in the plasma of fresh and stored butter, the amounts were greater in the non-washed butter, but the no. did not coincide with that in the milk protein. The total amount of amino-acid N increased with storage, but some of the acids occurred less in the stored non-washed butter. (12 references.) A. L. GROCHOWSKI.

Moisture dispersion in butter as determined by electrical resistance measurements. M. N. Hermann and S. H. Lombard (*S. Afr. J. agric. Sci.*, 1962, **5**, 439—449).—Resistance was measured using a Wheatstone bridge and tubular electrodes of stainless steel, the longer tube being filled with butter and the smaller tube embedded in it. The resistance provided a reliable indication of moisture content in well-worked butter. Poorly worked butter shows considerable variation within the sample. Salt has a slight effect on resistance. Changes in the dispersion of moisture in the butter during working are described. (15 references.) S. G. AYERST.

Colorimetric determination of hydrogen sulphide in cheese. S. Poznański (*Roczn. Technol. Chem. Żywnosci*, 1962, **9**, 17—27).—A new simple procedure, requiring only 10 g. samples, based on the Johnson and Nishita method and apparatus (cf. *Anal. Chem.*, 1952, 736) was developed for determinations of H_2S in cheese. To the weighed sample, water (20 ml.) and 12·5% by vol. H_2SO_4 (4 ml.) were added, N_2 was introduced at 150—200 ml./min., and the mixture was boiled 45 min. at 105° in a glycerol bath. The H_2S evolved was absorbed by the Johnson and Nishita reagent (*loc. cit.*) and determined by colorimetry with the use of *p*-aminodimethylaniline. The same apparatus was suitable for absorbing the H_2S in the Horton and Thomason $CdCl_2$ reagent (cf. *Anal. Chem.*, 1951, **23**, 1859) followed by indirect determinations of Cd bound as CdS by polarography or by the EDTA titrations. The accuracy of the colorimetric procedure with a Pulfrich colorimeter was 13·315 ± 0·133 μg. H_2S in a 10 g. sample of cheese. It could be improved by the use of photo-colorimeters. (16 references.) A. L. GROCHOWSKI.

Preparative separation of high molecular peptides of cheese. B. Lindqvist (*Sci. Tools*, 1962, **9**, No. 2, 13—15).—Gel filtration followed by measurement of u.v. absorption and changes of conductivity in the eluate gives a quick and simple means of coarse-fractionating protein–peptide–amino-acid mixtures. The combination of u.v.-photometer and conductivity meter, with continuous recording of the properties of the eluate, gives detailed information of the course of the gel filtration. U.v. and conductivity curves are given.
C. A. P.

Storage properties of dried baby milk. J. Pokorný, J. Písecký and R. Kohn (*Prům. potravin*, 1963, **14**, 139—143).—Samples of several popular sorts of dried baby milk, and a sample of the so-called 'humanised' milk, containing soya-bean oil, were stored for 9 months under current storing conditions, to study their stability.

Wode's benzidine tests were employed for determining the oxidation rate, and were sometimes combined with thiobarbiturate tests or with determination of colour changes, since methods based on peroxide no. failed to give reliable results. Durability of the 'humanised milk' is by 50% worse than that of current makes, but it can be reasonably expected that with improving technology, the storage properties will be improved too. (19 references.) J. S. B.

Microbiology of egg products. C. L. Heller (*Chem. & Ind.*, 1963, 228—229).—The microbiology of eggs and egg products is reviewed. Methods of manufacturing dried albumin and whole egg products are described. M. O'LEARY.

Detection and estimation of chlorinated pesticides in eggs. J. H. Onley and P. A. Mills (*J. Ass. off. agric. Chem., Wash.*, 1962, **45**, 983—987).—Mills' procedure (*ibid.*, 1959, **42**, 734) is modified by removal of interfering halogenated compounds of unknown composition; the chlorinated pesticides present in the sample can then be identified and determined by column or paper chromatography. Recoveries in the range 73—110% are reported.

A. A. ELDRIDGE.

Stable sweetened cream product. A. Tamsma (B.P. 889,251, 5.5.58. U.S., 6.5.57).—There is claimed a stable, pasteurised cream product consisting of milk fat (30—50%); non-fat milk solids (0·1—0·4 pt. per pt. of milk fat); sucrose (preservative); and water, the sucrose-in-water amounting to 60—65% of the product.

F. R. BASFORD.

Edible Oils and Fats

Detection of small quantities of polyglycerol-fatty acid esters in edible fats and foods containing them. J. Wurzinger and W. Gebauer (*Brot u. Gebäck*, 1962, **16**, 209—212).—The method depends on partial removal of glycerol from a prepared extract containing glycerol and polyglycerols by evaporation under vac., followed by separation of polyglycerols from remaining traces of glycerol by circular paper chromatography. Chromatograms on 2043b (Sch and Sch) paper are developed for 24 to 36 h. with water-saturated butanol in an atm. of NH_3, followed by spraying with ammoniacal-$AgNO_3$ and heating at 130°. The presence of polyglycerols is shown by the development of from two to four dark brown black rings outside the ring due to traces of glycerol. The method will detect 0·5% of polyglycerols in glycerol. E. C. APLING.

Effect of the inhibitor concentration on the oxidation rate in certain vegetable oils. C. Pietrzyk (*Roczn. Technol. Chem. Żywnosci*, 1962, **9**, 81—98).—Refined soya-bean oil, I no. 120·9, peroxides 0·015 mg. active O/g., refined rapeseed (colza) oil, I no. 101·9, peroxides 0·037 mg. active O/g., and partly oxidised linseed oil, I no. 167·3, peroxides 0·930 mg. active O/g. were treated with various concn. of commercial inhibitors. The latter included a mixture of 2- and 3-isomers of t-butyl-4-hydroxyanisole (Eastman Tenox BHA), 2,6-di-t-butyl-methylphenol (Eastman Tenox BHT) and nordihydroguaiaretic acid (NDGA). Optimal effect in the rapeseed oil was obtained with 0·06% by wt. concn. of either BHA or NDGA. Pro-oxidative effect was noted with higher concn. of these antioxidants. The BHT did not show pro-oxidative effect in above three oils, even at concn. as high as 5% by wt., and was classified as a 'strong' antioxidant. Mixtures (1 : 1) of BHA and BHT in the rapeseed oil controlled the oxidation according to the BHT concn., but the pro-oxidative effect of BHA was eliminated. The pro-oxidative effect of the higher concn. and the behaviour of antioxidant mixtures can be explained by the mechanism of the radical chain reactions. Equations relating the oxidation rate with the concn. of the antioxidants are presented, and the 'strength' and 'efficiency' factors are calculated. A. L. GROCHOWSKI.

Pro-oxidant action of propyl gallate during the autoxidation of soya-bean oil and of colza [rapeseed] oil. C. Pietrzyk (*Roczn. Technol. Chem. Żywnosci*, 1962, **9**, 29—46).—The effect of propyl gallate inhibitor (Eastman Tenox PG) added in various concn. to samples of soya-bean and rapeseed oils was investigated in the 53—100° temp. range of autoxidation. At concn. admissible for fat stabilisation only slight inhibitory effect on the rate of oxidation was observed. At concn. from 0·06 to 0·10% the lowest initial oxidation rate was observed, while at the higher concn. the rate increased. Detailed data are tabulated and discussed. The induction periods increased with the concn. of propyl gallate in the oil, but not in proportion to the initial rate of the peroxide formation. For rapeseed oil oxidised at 75 and 100° respectively the induction periods at 10^{-4} concn. of the inhibitor were 20 and 6 h. respectively, at 10×10^{-4} concn. 240 and 52 h., at 60×10^{-4} concn. 1200 and 92 h. respectively. The pro-oxidant action of propyl gallate at the higher concn. is explained by two different reactions with peroxide radicals, (*a*) forming active radicals and accelerating the oxidation, and (*b*) no active radicals are formed and the oxidation is inhibited. Kinetic computations are quoted supporting these assumptions. (24 references.) A. L. GROCHOWSKI.

[A] **Spectrophotometric microdetermination of peroxides in fats.** [B] **Microdetection of peroxides in fats.** A. Vioque and E. Vioque (*Grasas y Aceites*, 1962, **13**, 203—206, 211).—[A] A solution of the oil in 1 : 1 acetic acid–$CHCl_3$ (500 μl.) is treated with 0·1% aq. $FeSO_4(NH_4)_2SO_4$ (50 μl.) and 0·1% *NN*-dimethyl-*p*-phenylenediamine hydrochloride in 5 : 5 : 1 $CHCl_3$–AcOH–water (2·5 ml.). After leaving in darkness for 0·5 h. the extinction at 517 mμ is measured spectrophotometrically. The peroxide content is calculated with reference to a calibration curve obtained with a rancid soya-bean of known peroxide value.

[B] A spot on filter paper, from a solution of the oil, is treated with the $FeSO_4(NH_4)_2SO_4$ and *NN*-dimethyl-*p*-phenylenediamine reagents as above. A red coloration, intensifying on heating is obtained with peroxides. L. A. O'NEILL.

Physico-chemical studies on ground olive pastes. XVI. Measurement of rheological properties of the pastes. C. W. Brabender, G. Hahn, J. Martinez Moreno, C. Gómez Herrera and C. Janer del Valle (*Grasas y Aceites*, 1962, **13**, 197—202).—The rheological properties of ground olive pastes with and without a surfactant (a fatty acid diethanolamide) were studied with a Brabender Plastograph. (37 references.) L. A. O'NEILL.

Minor components in olive and sulphur olive oils. I. Alcoholic compounds obtained from the unsaponifiables of sulphur olive oil. J. Martel and J. Gracián (*Grasas y Aceites*, **13**, 212—215).—The thin-layer chromatograms of the unsaponifiables of two grades of olive oil, esterified S olive oil fatty acids and a S olive oil have been compared. The esterified oil gave a very simple chromatogram showing only three spots, due to hydrocarbons, sterols or highly polar compounds. The chromatograms of the other oils were more complex but showed minor differences. A well-defined spot from the S olive oil was studied in detail and found to result from a dialcohol, m.p. 208—210°, identical with a compound previously isolated from olive leaves (Power and Tutin, *J. chem. Soc.*, 1908, 891) and designated homo-olestranol. (16 references.)

L. A. O'NEILL.

Formation of antioxidative substances during the dry rendering of lard. A. Rutkowski and W. Korzeniowski (*Roczn. Technol. Chem. Żywnosci*, 1962, **9**, 69—79).—Literature data indicate that dry rendered lard, although containing more Fe (1·17 mg.-%), has better stability than the lard prepared by expulsion method containing only 0·84 mg.-% Fe. A series of tests on industrial open-vessel dry rendering proved that the stability of obtained lard increased when the rendering temp. was increased to 110—120°. It is assumed that antioxidative substances evolved on the formation of greaves penetrate into the lard and thus increase its resistance to autoxidation. The substances result from the 'non-enzymic browning' of the proteins. Additions of the greaves fat to lard obtained by cold extraction with light petroleum increased the stability of such lard. The antioxidants formed during the dry rendering were easily sol. in 96% EtOH and less sol. in 72% EtOH and 70% BuOH. Greaves de-fatted with light petroleum yielded more of the antioxidant on extraction with 96% EtOH. The stabilising effect appeared to be closely connected with the N content, and the 96% EtOH extracts of ordinary commercial lard and of good stability greaves lard contained 0·005 and 0·051% N respectively. The antioxidants are non-fat and unsaponifiable.

A. L. GROCHOWSKI.

Free fatty acids of cod oil. I. Anomalous composition by fatty acid chain length. R. G. Ackman, R. D. Burgher and M. L. Hughes. **Air blowing of cod-liver oil. II. Changes in fatty acid composition as determined by gas–liquid chromatography.** P. M. Jangaard, R. G. Ackman, R. D. Burgher and M. L. Hughes (*J. Fish. Res. Bd Can.*, 1962, **19**, 1081—1084; 1963, **20**, 89—94).—I. Examination of the fatty acid composition of a Newfoundland cod oil by gas–liquid chromatography indicated that the proportion of docosahexaenoic acid, relative to docosenoic acid was much lower in the free fatty acids (FFA) than in the neutral lipids. The proportion of myristic acid in the cod oil was significantly higher in the FFA. The same general conclusions were drawn from three other cod oils from other sources. These discrepancies were studied quant. by chromatographic chain length analyses of the hydrogenated methyl esters of the fractions. Specific enzyme and bacterial actions may be responsible. (10 references.)

II. The effect of air blowing at 80 and 130° on the major component fatty acids of cod-liver oil was determined. Saturated acids are unchanged after air blowing at 130° for 6 h., the amount of monounsaturated acids decreases slightly (1—3%) and the major highly unsaturated acids, eicosapentaenoic and docosahexaenoic, disappear. (10 references.) E. M. J.

Colorimetric microdetermination of hydroxyl value. E. Vioque and M. P. Maza (*Grases y Aceites*, 1962, **13**, 207—210).—The sample (1 to 2·5 mequiv. of OH) is acetylated at 90 to 100° with acetic anhydride in pyridine and the excess reagents removed with a current of N_2. The acetylated product is heated with an alcoholic hydroxylamine solution (prepared by mixing alcoholic solutions of NaOH and $NH_2OH{\cdot}HCl$ and centrifuging) (0·2 ml.) at 60 to 70° for 5 min., the mixture cooled, and an alcoholic solution of $Fe(ClO_4)_3$ (containing a little HNO_3) (3 ml.) added. The absorption at 520 mμ due to the ferric-hydroxamic acid complex is then measured spectrophotometrically. The hydroxyl value is calculated with reference to a calibration curve obtained with pure cholesterol. If the material does not contain ester groups this can be done directly, but if ester groups are present it is necessary to work on the difference between the acetylated and unacetylated material. The method is satisfactory for alcohols, hydroxyacids, partial esters, etc., containing primary or secondary OH groups. L. A. O'NEILL.

Purification of fats. A.-B. Pellerins Margarin-Fabrik, Assee of L. O. Bergman (B.P. 887,885, 20.9.60. U.S., 21.9.59).—Fat (hardened whale oil, hardened groundnut oil, hardened cottonseed oil, tallow or lard), in vac.-dried anhyd. state, is kept in contact with 0·025—0·3 wt.-% of conc. H_3PO_4 at 60—90°/~ 6 mm. for ≤5 (≤30) min., and is then refined with aq. alkali, to give a purified product suitable for manufacture of margarine. F. R. BASFORD.

Meat and Poultry

Microbiology of raw materials for the meat industry. E. J. Dyett (*Chem. & Ind.*, 1963, 234—237).—Methods of disinfecting pig pens and sterilising meat knives are described together with microbiological testing techniques and standards for raw meats, gelatin, spices and casings. The function of the bacteriologist in food factory control is discussed. M. O'LEARY.

Collagen content and its relation to tenderness of connective in two beef muscles. S. J. Ritchey, S. Cover and R. L. Hostetler (*Food Technol.*, 1963, **17**, No. 2, 76—79).—*Longissimus dorsi* contained less collagen N than did *biceps femoris* muscle in raw and in steaks cooked to internal temp. of 61 and 80°. Rates of conversion of collagen to gelatin were similar. Large variations among animals were observed in collagen N content and in the % transformed to gelatin during cooking. Trends were toward tendering with increasing temp. for both muscles as measured by panel scores and collagen content. The hydroxyproline content of alkali-insol. autoclave-sol. filtrate was used as a measure of collagen. (28 references.) E. M. J.

Subjective and objective evaluations of prefabricated cuts of beef. M. M. Fielder, A. M. Mullins, M. M. Skellenger, R. Whitehead and D. S. Moschelte (*Food Technol.*, 1963, **17**, No. 2, 95—100).—The effect of grade (**I**) on the palatability of eight frozen prefabricated beef cuts from 32 carcasses was studied. Prefabrication of carcasses minimised **I** on the values tested. **I** was significant on all chemical data but not when data from prime carcasses were removed. Juiciness appeared to be the palatability attribute most affected. Correlations were calculated between subjective and objective measurements of flavour, juiciness and tenderness. [Carcasses are processed into cuts according to tenderness of muscles.] (14 references.) E. M. J.

Use of a slice-tenderness evaluation device with pork. R. Kulwich, R. W. Decker and R. H. Alsmeyer (*Food Technol.*, 1963, **17**, No. 2, 83—85).—The device is described and illustrated. The multiple-correlation coeff. for the relation between slice-tenderness evaluator shear- and puncture-force readings, parallel to muscle fibre orientation and taste panel tenderness scores for cooked pork *longissimus dorsi* muscle samples from 61 pigs, was −0·79. This was very close to the −0·80 simple correlation coeff. obtained for the relation of Warner–Bratzler shear and taste-panel scores. E. M. J.

Effect of bleed time prior to scald and refrigerated storage on bacterial counts in the axilliary diverticula of the interclavicular air sac of chickens. F. R. Tarver, jun., and K. N. May (*Food Technol.*, 1963, **17**, 80—82).—Generally, bacterial counts for bleed times of 90 sec. and less (prior to 120-sec. scald) were not significantly different from each other; similarly for counts for 120-sec. or longer bleed times. Bacterial counts of the shorter bleed times were significantly higher than those for the longer bleed times. The controls (bled until lifeless and not scalded) had counts that were significantly lower than any bleed time of the scalded birds. The counts for the various bleed times were generally higher at the end of the second week of refrigerated storage than at the end of the first week. E. M. J.

Periodate oxidation of gelatin extracted from bone. R. B. Aronson (*Dissert. Abstr.*, 1962, **23**, 1184).—The gelatin was oxidised in 0·1N-NaOH with $NaIO_4$ and 65% of the hydroxylysine (**I**) was destroyed in 1 h. The reaction is not a simple first- or second-order one but consisted of two first-order reactions, one 14 times faster than the other. Corrections for possible HCHO formation did not give a first-order graph and this suggests that the cause of the anomaly is not carbohydrates. It is suggested that **I** exists in two forms: one with a reactive group in a side-chain and the other with the reactive group blocked. F. C. SUTTON.

Gelatin composition. General Foods Corp. (B.P. 888,643, 3.5.60. U.S., 11.5.59).—Gelatin metaphosphate (prepared from gelatin and KPO_3) is treated, at ≤40°, with an active cationic prosthetic group comprising a metal (Ca, Al, Ag), an amine or an electropositive org. group. The product with Ca is practically tasteless and goes into solution much more rapidly than the untreated gelatin. J. M. JACOBS.

Fish

Apparatus for measuring the energy input in cutting fibres of fish muscle. H. Buttkus (*J. Fish. Res. Bd Can.*, 1963, **20**, 183—186).—The construction and operation of an apparatus with which the resistance of muscle fibres to a cutting force can be measured is described. A quant. comparison of the toughness of different samples can be made. Measurements on a model system of paraffin wax show a variation of ±5%. With muscle fibres of raw and cooked salmon, lingcod and beef, the variation in these measurements was sometimes as high as ±20%. (12 references.) E. M. J.

Red and white muscle of fish in relation to rigor mortis. H. Buttkus (*J. Fish. Res. Bd Can.*, 1963, **20**, 45—58).—By comparison with white muscle, rigor contraction and isometric rigor tension in red muscle in lingcod were about three times as great. The rate of contraction of the red muscle depends on temp. and on the O_2 concn. in the surrounding atm. The elastic modulus of the red muscle increased with increasing post-mortem time. The max. effects of contraction, tension and elasticity coincided with the onset of rigor mortis. Stiffening of a fish with the onset of rigor mortis is not due to contraction or tension development of the muscles but to their changing mechanical properties; a measure of these changes is the elastic modulus. Electron micrographs showed that red muscle in rested fish contained 1—3 times more glycogen than did white muscle. (24 references.) E. M. J.

Improved method for the preparation of fish protein concentrate from cod. H. E. Power (*J. Fish. Res. Bd Can.*, 1962, **19**, 1039—1045).—A high-quality fish protein concentrate can be produced from fish fillets by extraction with suitable isopropanol–water mixtures. Such a substance was produced from skinned cod fillets, on a pilot plant scale. The yield of fish protein with 50 lb. lots of fillets and batch processing was between 12 and 15%, the white concentrate being odourless and tasteless, soft in texture, as wheat flour. Proximate analysis of a typical sample has the following composition: moisture 4, ash (dry basis) 5, lipid 0·033, protein (N × 6·25) 96% (dry basis). E. M. J.

Bound nucleotides of freshly frozen and severely denatured frozen lingcod muscle. N. Tomlinson and S. E. Geiger (*J. Fish. Res. Bd Can.*, 1963, **20**, 187—194; cf. Connell, J.S.F.A. Abstr., 1963, i, 160).—The bound nucleotides of lingcod (*Ophiodon elogatus*) muscle were examined in relation to the denaturation of muscle proteins during frozen storage. No difference was found in the quantity of adenine nucleotides (adenosine diphosphate) bound in washed muscle residues from freshly frozen (pre-rigor) and in those from muscle severely denatured during frozen storage. There was somewhat less adenosine triphosphate bound in the extracts from the severely denatured muscle than in those from the undenatured frozen muscle. Heating lingcod muscle released bound nucleotide. (36 references.) E. M. J.

Chemical indexes of decomposition in perch stored in natural ice and in chlortetracycline (CTC) ice. F. Hillig, L. R. Shelton, J. H. Loughrey, S. Bethea and C. M. Campbell (*J. Ass. off. agric. Chem., Wash.*, 1962, **45**, 922—951).—The decomposition of ocean perch has been followed by determinations of volatile acid, formic and acetic acids, volatile bases, volatile amines, and trimethylamine. Whole fish were stored in ice or ice containing tetracycline; fillets were dipped in brine or in brine containing tetracycline. Extensive data are presented in tables and graphs. The use of tetracycline permits the development of chemical indexes of decomposition. A. A. ELDRIDGE.

Chemical indexes of decomposition in haddock stored in natural ice and in chlortetracycline (CTC) ice. F. Hillig, L. R. Shelton,

J. H. Loughrey, S. Bethea and C. Campbell (*J. Ass. off. agric. Chem., Wash.*, 1962, **45**, 951—978).—Chemical data similar to those relating to ocean perch (see previous abstract) are presented. The fish were stored under similar conditions, and similar conclusions were reached. A. A. ELDRIDGE.

Colour changes in black coloured fish roe products and their food-law significance. J. Wurzinger (*Dtsch. Lebensmitt Rdsch.*, 1962, **58**, 357—360).—Fish roes coloured with Brilliant Black BN gradually change colour on storage from black, through grey-brown to (when stored at 37°) dirty orange. The colour extracted by 30% acetic acid solution varies, from greyish-green from fresh roes, to red or yellow-brown after storage, and the colours detectable by paper chromatography undergo parallel changes suggesting the presence of colours prohibited under German food law. Preserved fish roes show no visible colour change but do show alteration of the colour extracted by acetic acid solution, which can therefore be used as a simple criterion of freshness. E. C. APLING.

Zinc and zinc-65 in shellfish of Fishers Island Sound and its estuaries. B. W. Fitzgerald (*Dissert. Abstr.*, 1962, **23**, 1522).—The levels of zinc-65 in shellfish were studied. Oysters were placed in trays of contained seawater to which had been added various salts of Zn labelled with ^{65}Zn. The effect of the different anions upon the rate and extent of Zn uptake was then followed by tracer techniques. The results obtained indicated that the levels of ^{65}Zn in oysters in this area are low in comparison with those obtained in samples from the West Coast of the U.S.A. Complexation of the Zn will, however, considerably reduce the rate and extent of uptake. F. C. SUTTON.

Spices, Flavours, etc.

Preparation of aroma concentrates. V. Special aromas. A. M. Burger (*Riechstoffe u. Aromen*, 1963, **13**, 41—45).—The botanical varieties of coffee, various identified aroma substances and applications of natural and synthetic aroma concentrates are surveyed. Cacao seeds and their processing and cocoa essences and distillates are reviewed. Tea aroma is also mentioned. (20 references.) M. SULZBACHER.

Physical methods for analysing ethereal oils. II. Colorimetry. S. S. Nigam and G. L. Kumari (*Riechstoffe u. Aromen*, 1963, **13**, 1—11).—A review of the colorimetric methods for detecting or determining citral, geraniol, linalool, 1,8-cineol, eugenol or thymol in ethereal oils, based on treatments with specific reagents (described) to develop a characteristic colour, and detecting the colour or measuring its intensity. (11 references.) H. L. WHITEHEAD.

Gas chromatography using capillary column units for flavour investigation. R. Self, D. G. Land and J. C. Casey (*J. Sci. Fd Agric.*, 1963, **14**, 209—220).—Modifications of techniques (which are suitable for relatively conc. samples), to be applied to very low vapour concn., e.g., vegetable flavours, are discussed. The progress made in applying capillary apparatus to flavour problems is described. Details with diagrams of construction and operation are given. (21 references.) E. M. J.

Colouring matters

Carotenoid preparations. F. Hoffmann-La Roche & Co. A.-G. (B.P. 887,883, 5.9.60. Switz., 8.9.59).—A solution of a carotenoid in a volatile, water-insol-solvent (low mol. halogeno-hydrocarbon, e.g., chloroform or methylene dichloride) is emulsified in an aq. solution of a swellable colloid (gelatin, gum arabic, dextrin or polyvinyl alcohol) optionally containing a plasticiser, then solvent is removed, and the residual suspension is spray-dried (if desired), to give a prep. suitable for use as colour additive in foodstuffs. F. R. BASFORD.

Preservatives

Separation, identification and estimation of aromatic food preservatives and sorbic acid by paper chromatography and ultra-violet spectrophotometry. T. Höyem (*J. Ass. off. agric. Chem., Wash.*, 1962, **45**, 902—905).—Benzoic acid, methyl, ethyl, propyl and butyl *p*-hydroxybenzoate and sorbic acid can be separated by descending paper chromatography with a solvent composed of n-butanol, NH_3 and water, the positions of the spots being determined by photographic contact prints. The spots are then cut out, the org. substances extracted, and their extinction measured. R_F values and spectral characteristics are tabulated. A. A. ELDRIDGE.

Preserving eggs with polyvinyl alcohol coating. A. Šomogyi (*Prům. potravin*, 1963, **14**, 146—147).—If first-class fresh eggs are used, the method shows advantages especially for shorter storage periods up to 3—4 months. The principal requirement is the use of eggs not older than 24 h., storage at constant temp. 14—15° or below, at R.H. 80—85%. The advantages are lower losses of wt. by drying, prevention of CO_2 escape, non-acceptance of extraneous odours, and conservation of a relatively good taste. The method allows the preservation of eggs even with damaged egg shell, provided the inner membrane is intact. J. S. B.

Preserving or sterilising preparations. CIBA Ltd. (B.P. 887,685, 25.9.59. Switz., 25.9.58).—A disinfecting and preserving agent for medicaments (vaccines etc.) and foodstuffs comprises β-*p*-chlorophenylethanol (0·4—2·0%). H. S. R.

Food Processing, Refrigeration

Influence of surface pasteurisation and chlortetracycline [CTC] on bacterial incidence on fryers [chickens]. L. E. Dawson, W. L. Mallmann, D. G. Bigbee, R. Walker and M. E. Zabik (*Food Technol.*, 1963, **17**, No. 2, 100—103).—Pasteurisation at >140°F for longer than 1 min. gave partial cooking. Bacterial counts for frying chickens pasteurised 5—10 min. at 135—140°F and chilled in slush ice containing CTC 10 p.p.m. were approx. 100 times less than on control birds. Acceptability was extended about 1 week longer than in controls. E. M. J.

Sulphuring of dry sultana raisins. Amount of total sulphur dioxide absorbed by the raisins in relation to different sulphuring conditions and its effect on the colour of the raisins. C. D. Exarchos and A. A. Moïsidis (*Bull. technol. Inst. Plant Prod., Athens*, 1962, No. 2, 48—58).—Generally in Greece the seedless raisins grown there are bleached by exposing the raisins under rather empirical conditions to the fumes from burning S. The present work shows that better results and control can be obtained by (i) using a liquid SO_2 supply giving a higher SO_2 concn., (ii) early treatment, e.g., within 1 month of harvest and (iii) by continuous agitation of the raisins during sulphuring. Under these conditions effective sulphuring could be obtained in 20 to 30 min. which economically is more favourable than the present much longer process. (From English summary.) W. ELSTOW.

Spoilage in Indian canned fruits and vegetables. M. S. Subba Rao and D. S. Johar (*J. sci. industr. Res.*, 1962, **21D**, 427—428).—Spoilage in cans of fruit and fruit products is caused entirely by H_2-swell (**I**), but in cans of vegetables and their products it is caused variously by infection from cooling-water (43), autosterilisation (30), heat-resistant organisms (15) and overfilling or incomplete vac. (12%). Causative micro-organisms are *Bacillus subtilis* and *B. firmus*; **I** is minimised by ensuring high vac. and can storage at low temp. (13 references.) W. J. BAKER.

Freeze dehydration of foods. I. II. S. A. Goldblith, M. Karel and G. Lusk (*Food Technol.*, 1963, **17**, No. 2, 21—26, No. 3, 22—28).—I. Problems associated with the freeze dehydration of foodstuffs are reviewed covering the following: raw materials; physical and chemical properties of foods the changes of which influence quality of product; microbiological aspects; food engineering problems; and process parameters that affect food quality.

II. Effect of changes in the process characteristics on the quality of freeze-dried sea-food products in a pilot-plant freeze dryer were studied with shrimp and salmon, concerning (*a*) temp. programme during the drying cycle, (*b*) cooking time and temp., (*c*) temp. of the freezing medium (rate of freezing), (*d*) temp. of rehydration water. Measurements of chemical changes were also useful, e.g., the oxidative reactions in the carotenoid pigment astacene (3, 4, 3′, 4′-tetraketo-β-carotene). (21 references.) E. M. J.

Freezing fruit for jam-making. Anon. (*Mod. Refrig.*, 1963, **66**, No. 778, 37).—The use of a 70,000 cu. ft. freezer room cooled to 0—5°F by eight high-capacity forced-air coolers is described. Although essentially designed for freezing, they can also be operated at 34—36°F for short-term storage, specially for strawberries which are made into jam with little delay. C. V.

Dehydrated food products. A. I. Morgan, jun. (B.P. 889,064, 26.10.59. U.S., 30.10.58).—A dehydrated food product (fruit and vegetable juice, milk prep., coffee extract, etc.) is obtained in improved physical form without the use of expensive equipment, by adding a foam stabiliser (e.g., monoglyceride of a fatty acid or hydrophilic colloid, such as egg albumin) to a conc. aq. dispersion of the product, then forming a foam, and heating the foam at 49—105°/≯1 atm., to produce a dehydrated, water-dispersible solid. F. R. BASFORD.

Packaging

Fungistatic packaging materials based on sorbic acid and calcium sorbate. E. Lück (*Dtsch. LebensmittRdsch.*, 1962, **58**, 353—375).—Paper treated with sorbic acid or Ca sorbate (2 g. per sq. m.; fixed with CMC solution) was used for the wrapping of cheese and margarine, and the sorbic acid content of 3 mm. layers of the foodstuffs were determined after storage for 3, 6 and 20 days at 28°. Diffusion of the preservative into the food was much slower with the Ca sorbate than with the free acid, and after 20 days was undetectable in layers of cheese over 6 mm., and of margarine, over 12 mm., beneath the surface. No off-flavours were produced and the use of these treated packaging materials is considered physiologically unobjectionable. (19 references.) E. C. APLING.

Relationship between the permeability and solubility characteristics of non-porous packaging materials: plastic films and water vapour. R. W. Pecina (*Dissert. Abstr.*, 1962, **23**, 1635—1636).—A systematic relationship exists between the water vapour permeability and solubility of a plastic packaging film at constant temp. over a useful R.H. range but does not exist at constant R.H. over a useful temp. range. An empirical recommended test procedure for predicting both the temp. and humidity effect on water vapour permeability, when the water vapour solubility characteristics for the packaging film are known, is given. F. C. SUTTON.

Dark discoloration of canned all-green asparagus. I. Chemistry and related factors. II. Development of a new tin plate for its control. H. H. Hernandez and D. C. Vosti (*Food Technol.*, 1963, **17**, No. 1, 95—99, 100—102).—I. Dark discoloration of all-green asparagus brine is dependent on the type of container corrosion and this is due to unknown systems in the asparagus. Discoloration does not occur if sufficient stannous Sn is in solution and this fact is related to all effects of other factors. (13 references.)

II. A new electrolytic tin plate, No. 135-25, is now available to can manufacturers offering a means of avoiding dark discoloration. The heavier coated surface becomes the inside of the can and affords protection from product. The lighter-coated surface is applied to prevent exterior rusting. As selective chemical critical treatment chromic acid gave satisfactory results. E. M. J.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Products of glycerylphosphatide hydrolysis. C. Ravazzoni, G. Donelli and R. Valerio (*Chim. e Industr.*, 1962, **44**, 1380—1382).—Lysolecithin and glycerylphosphorylcholine are discussed from the standpoint of their presence in nature and of their importance as water-sol. intermediates in lipid metabolism. Methods for prep. and quant. determination of L-α-glycerylphosphorylcholine are discussed. Paper- and thin-layer chromatography data are given; the latter method is particularly useful for following the prep. progress. (24 references.) C. A. FINCH.

Biosynthesis of protein-vitamin concentrates from petroleum. A. Champagnat, C. Vernet, B. Lainé and J. Filosa (*Nature, Lond.*, 1963, **197**, 13—14).—Work on obtaining protein–vitamin concentrates from petroleum by micro-organisms is reviewed. The composition of a concentrate obtained is given in detail and compared with the content of proteins and vitamins in other basic foodstuffs. S. A. BROOKS.

Amino-acid composition of leaf proteins. A. C. Chibnall, M. W. Rees and J. W. H. Lugg (*J. Sci. Fd Agric.*, 1963, **14**, 234—239).—The difficulties associated with a study of this kind are discussed. A realistic picture of the essential amino-acid composition can be obtained by column analysis of an acid hydrolysate of dried leaf material (whole protein prep.), the S-containing amino-acids, tyrosine and tryptophan, being determined by more appropriate procedures. This dried leaf material from which all sol. non-protein products have been removed can be used with confidence for the determination of the nutritive value of the leaf proteins by amino-acid analysis. (15 references.) E. M. J.

Vitamin-containing compositions. Commercial Solvents Corp. (Inventor: A. Rosenberg) (B.P. 887,813, 12.3.58).—A molten mixture of one or more oil-sol. vitamins (A or E), high-melting fat, and 0·2—15% of hot well oil (unrefined water-insol. portion of condensate obtained in the deodorisation of vegetable oils by high-vac. steam distillation) is converted into granules to give a stable product suitable for incorporation into feedstuffs. F. R. BASFORD.

Unclassified

Infra-red, ultra-violet and visible absorption spectra of some USP and NF reference standards and their derivatives. A. L. Hayden, O. R. Sammul, G. B. Selzer and J. Carol (*J. Ass. off. agric. Chem., Wash.*, 1962, **45**, 797—900).—The i.r., visible, and/or u.v. curves are presented for 207 compounds, together with data relating to structure and dispersion medium or solvent. The compounds are treated alphabetically and an index is provided. A. A. ELDRIDGE.

Determination of microgram quantities of tin in foods. M. H. Thompson and G. McClellan (*J. Ass. off. agric. Chem., Wash.*, 1962, **45**, 979—982).—Luke's method (*Analyt. Chem.*, 1956, **28**, 1276) is adapted for use with foods containing relatively small quantities of interfering metals. Recoveries of tin were 83—112% (average 100%) from cooked shrimp. A standard mean deviation of 1·4 μg. and an average deviation of 1·2 μg. per aliquot containing 20 μg. of tin were obtained. A. A. ELDRIDGE.

Recommended methods of analysis of pesticide residues in foodstuffs. Determination of demeton-methyl residues in fruits and vegetables. Report by The Joint Demeton-methyl Residues Panel (*Analyst*, 1962, **87**, 485—492).—A sample (50 g.) is macerated with methanol–water (1 : 1) and the filtered extract, evaporated to ~5 ml., is applied to a column of active C and $CHCl_3$ (prep. described). The evaporated eluate is heated to fuming with HNO_3, $HClO_4$ and HCl and after addition of HNO_3 and after addition of water. The aq. solution, neutralised by addition of aq. NH_3 and boiling, is treated with NH_4 molybdate solution, the vol. adjusted to 13 ml. and extracted with 9 ml. of isobutanol–benzene (1 : 1), the separated org. layer being washed with N-H_2SO_4, transferred to a stoppered cylinder with ethanol–H_2SO_4 to 9·6 ml. and 0·4 ml. of $SnCl_2$ solution added. The extinction is measured at 730 mμ against a blank and referred to a standard graph. The application of the method of Laws and Webley (*Analyst*, 1962, **86**, 249) essentially similar to the recommended method is described. A. O. JONES.

Gas chromatography of food volatiles—an improved collection system. I. Hornstein and P. F. Crowe (*Analyt. Chem.*, 1962, **34**, 1354—1356).—A system whereby the volatile products from foods were collected in a refrigerated (liquid N_2) stainless steel or Cu coil, connected directly to the gas chromatographic column, is described. Both columns are packed with 25% Castorwax on 30- to 60-mesh Chromosorb W, and the sweep gas used is N_2. After sample collection the temp. programming is carried out over the range 40—125°. The coil appears more efficient for trapping volatiles than the usual U-tube system, and no gas transfer systems or heated injection points are needed. R. A. HENDEY.

Isolation and description of pectinase-inhibiting tannins of grape leaves. W. L. Porter and J. H. Schwartz (*J. Fd Sci.*, 1962, **27**, 416—418).—The tannin was isolated and purified by pptn. with caffeine and recovery by $CHCl_3$ extraction. The substance was pale tan and on freeze-drying was light and bulky. It is a condensed tannin of high mol. wt. producing a large % of phlobaphenes on acid treatment. The sol. portion contained gallic acid and glucose in small amounts. (12 references.) E. M. J.

Microscopical detection of commonly used dry mixtures of thickeners. A. T. Czaja (*Z. LebensmittUntersuch.*, 1962, **117**, 499—513).—Descriptions, with photomicrographs, are given of the microscopical appearance of eight types of thickeners under polarised light and after treatment with appropriate stains or reagents. P. S. ARUP.

Chemistry of whale products. L. C. Surmon and M. F. Ovenden (*S. Afr. industr. Chem.*, 1962, **16**, 62—72).—The following are reviewed: species and habits of whales, organisation of catch, techniques and subsequent processing of raw materials obtained, chemistry and commercial utilisation of oils (commercial and sperm), whale meat and whale-liver oil. A. S. CARMICHAEL.

Coating composition for articles of food. Dow Chemical Co. (Inventors: L. E. Patten and H. C. Kelly) (B.P. 875,855, 4.8.59. Addition to B.P. 832,449; J.S.F.A. Abstr., 1961, i, 249).—The composition is a mixture, adapted to be applied as a hot melt, of (1) 20—50% by wt. of an ethyl cellulose having OEt content 47·5—50% and a viscosity of 6—200 cP, as determined in a 5% by wt. solution in an 80 : 20 mixture, by vol., of toluene and ethanol; (2) 15—68% of a refined mineral oil having a Saybolt viscosity at 100°F of 80—400 units; (3) >5% up to the max. compatible quantity of a wax constituent melting >100°F and (4) 10—25% by wt. of a colourless, odourless, non-toxic plasticiser for the cellulose ether (e.g., butyl phthalyl butyl glycollate). E. ENOS JONES.

Food. Battelle Development Corp. (Inventors: H. A. Sorgenti, H. Nack and G. F. Sachsel) (B.P. 888,649, 7.6.60).—A fast, greaseless method of treating (cooking) food (meat, fish, poultry, nuts, vegetables, coffee, and, especially, potatoes, parched sweet corn, shrimp, onion rings and coffee) comprises immersing the food in a fluidised bed of solid, discrete, non-toxic particles. Thus, potatoes sliced to a thickness of 25 slices per in. are coated with potato flour, then immersed in a fluidised bed of NaCl at 165° during 165 sec., to give a crisp, evenly cooked product. Cooling of food may be effected likewise. F. R. BASFORD.

3.—SANITATION, WATER, etc.

Microbiological aspects of one-trip glass bottles as used by the carbonated beverage industry. H. E. Korab (*Food Technol.*, 1963, **17,** No. 1, 108—109).—The bottles removed from the discharge end of the 'lehr' (heated tunnel in which temp. of bottles is lowered from 1100°F to near room temp.) in the glass factory were of high sanitary quality. With reasonable care they could be used directly, requiring at most an air or water rinse to remove possible cardboard fragments or dust contamination. The bottles tested would pass dairy standards. E. M. J.

Public-health aspects of handling animal products in the tropics. W. W. Sadler (*Food Technol.*, 1963, **17,** No. 2, 36—38, 41).—Diseases that may develop in human beings, from meat, meat animals and meat dishes are discussed. E.g., of eight tables given, III categorises prevalence of meat-borne disease agents as contaminants of meat. Basic factors in preventing meat-borne disease are sanitation, inspection and preservation. E. M. J.

Estimation of pyrethrins. A. Brierley and N. C. Brown (*Soap, N.Y.*, 1962, **38,** No. 10, 105, 107, 109, 111, 121).—The chief methods employed are discussed and compared; the occurrence of 'false' pyrethrins is specially examined. If these are removed on an alumina chromatogram, the results show that the esters of chrysanthemic acid present in pyrethrin oleoresin amount to ~70% of those given by the Pyrethrum Board of Kenya (PBK) method or 90% of those obtained by the Association of Official Agricultural Chemists (AOAC) technique. Further work to clarify the position is in progress. (14 references.) C. V.

Significance of the epoxidation of the isomeric insecticides aldrin and isodrin by the adult housefly *in vivo*. G. T. Brooks, A. Harrison and J. T. Cox (*Nature, Lond.*, 1963, **197,** 311—312).—The presence of atm. O_2 was required for epoxidation of aldrin and isodrin by the adult housefly *in vivo* but penetration of the applied insecticide was not affected by anoxia. These two insecticides possess lower intrinsic toxicities than do the corresponding epoxides, dieldrin and endrin. S. A. BROOKS.

Novel insect repellents. G. J. Baker (*Soap, N.Y.*, 1962, **38,** No. 12, 111, 114, 149, 152—153).—3-Chloropropyl n-octyl sulphide (**I**) and 2-hydroxyethyl n-octyl sulphide (**II**) are specially discussed. **I** is to be used at 0·2—1·0% concn. to protect animals from houseflies. **I** and **II** are readily sol. in org. solvents. The composition of various sprays is given and comparative repellency tests, both in laboratory and field, gave promising results. Mammalian toxicity is low. C. V.

Cumulative action of insecticides on adult mosquitoes. A. B. Hadaway and F. Barlow (*Ann. appl. Biol.*, 1962, **50,** 633—637).—Dieldrin, DDT, γ-$C_6H_6Cl_6$, malathion, diazinon and Sevin were all cumulative to some extent in their action on adult mosquitoes, *Aedes aegypti*. Mosquitoes receiving a sublethal dose of each insecticide were more susceptible to it 48 h. later than were untreated mosquitoes from the same population. Dieldrin was the most, and γ-$C_6H_6Cl_6$ the least, effective in this respect. A. H. CORNFIELD.

Joint action of insecticides on adult mosquitoes. A. B. Hadaway, F. Barlow and C. R. Turner (*Ann. appl. Biol.*, 1962, **50,** 639—648).—The responses of female mosquitoes (*Aedes aegypti* and *Anopheles stephensi*) to a mixture of two organo-P insecticides (malathion and diazinon) were the same as those to an equiv. dose of either insecticide, indicating that the two materials have similar modes of action. The responses of the mosquitoes to all the other mixtures used, malathion and a carbamate (Sevin), malathion and chlorinated hydrocarbons (DDT or dieldrin), Sevin and DDT or dieldrin, and DDT and dieldrin were lower than expected for similar action, but were higher than the sum of the responses to the same doses of each compound applied separately. Probably the compounds in each of these mixtures do not act independently. A. H. CORNFIELD.

Larvicide tests with colony-reared *Culicoides variipenis*. R. L. Harris and R. H. Jones (*J. econ. Ent.*, 1962, **55,** 575—576).—Insecticides (18) were tested in the laboratory by comparing larval mortality after 24 h. Chlorinated hydrocarbons were more effective than org. P compounds. Heptachlor and aldrin were the most toxic and sevin, the least toxic, was 300 times less effective. C. M. HARDWICK.

Lindane and BHC in egg yolks following recommended uses for louse and mite control. G. W. Ware and E. C. Naber (*J. econ. Ent.*, 1962, **55,** 568—570).—Lindane residues were found in egg yolks from all treated pens and the residues (mostly <1 p.p.m.) persisted for 14 days after removal of hens from the treated surfaces. Leg dips caused barely detectable residues. Egg production was unaffected except in an improperly sprayed pen. C. M. HARDWICK.

Quantitative gas chromatography of insect repellant mixture M-1960. F. Acree, jun., and M. Beroza (*J. econ. Ent.*, 1962, **55,** 469—471).—The repellant contained 2-butyl-2-ethyl-1,3-propanediol, *N*-butylacetanilide and benzyl benzoate. It was analysed quant. by gas chromatography on an 8 ft. column containing 5% Dow-Corning Silicone-550 on Haloport F at 225°. Dil. samples did not give as accurate a result as did conc. samples. Extracts of cloth samples were within acceptable limits. C. M. HARDWICK.

Bait composition and rodenticidal activity of barium carbonate. M. K. Krishnakumari, K. Krishnamurthy and S. K. Majumder (*Ann. Biochem.*, 1963, **23,** 5—8).—Increase in the protein concn. of bait was found to decrease the rate of mortality in albino rats caused by $BaCO_3$ and increase the death time. Effective baits had less than 10% protein. (11 references.) S. A. BROOKS.

Brackish water sources for irrigation along the eastern seaboard of the United States. M. H. Gallatin, J. Lunin and A. R. Batchelder (*U.S. Dep. Agric. agric. Res. Serv. Prod. Res. Rep.*, 1962, No. 61).—Brackish waters are considered to have a great potential for use as irrigation water provided sufficient knowledge of their salt content and chemical composition is available at time of irrigation. Studies on samples from four sites showed that salt content is chiefly influenced by climate, tides and mechanical soundness of dammed structures. The Na-adsorption-ratio (SAR) $(Na^+/\sqrt{Ca^{2+}+Mg^{2+}}/2$, where concentrations are expressed as mequiv. per l.) is considered to be a guide to the quality of a water. For waters of equal total salt content, one having a high SAR value is more hazardous than one having a low value. A graph relating electrical conductivity (EC) and SAR, prepared from determined EC and calculated SAR values of several dilutions of a synthetic sea water, was shown to facilitate estimation of the SAR values of waters from their EC values, provided the latter exceeded 1·5 mmho/cm. M. O'LEARY.

Development of water quality criteria for aquatic life. C. M. Tarzwell (*J. Wat. Pollut. Control Fed.*, 1962, **34,** 1178—1185).—A review article concerning parameters which should be fixed in order that a water may be evaluated for its toxicity to aquatic life. B. F. FULLAN.

Progress report on water quality criteria. E. L. Bean (*J. Amer. Wat. Wks Ass.*, 1962, **54,** 1313—1331).—A report from Task Group 2225M. Various factors in the production of ideal quality water, desirable characteristics and methods of analysis are discussed and an analytical procedure is detailed. (24 references.) B. F. FULLAN.

Seasonal variation in properties of aqueous humus. M. A. Shevchenko, P. B. Barashenkov and V. P. Chupova (*Ukr. khim. Zh.*, 1962, **28,** 403—409).—The seasonal alteration of content, coloration, oxidative potential, degree of hydrophily and degree of dissociation of the humus substances in Dnieper and Desna river waters are described. The substances are of soil origin, and the first is more coloured and less hydrophilic than the second. G. F. PENNY.

Free available chlorine residuals for small non-public water supplies. E. R. Baumann and D. D. Ludwig (*J. Amer. Wat. Wks Ass.*, 1962, **54,** 1379—1388).—Where the physical and biological character of raw water fluctuates rapidly and where constant supervision is not possible, superchlorination is recommended. For small water supply installations high Cl residuals are recommended. The use of Coxsackie virus as a criterion of chlorine resistivity in the disinfection of individual water supplies is suggested in the place of coliforms. (15 references.) B. F. FULLAN.

Water, wastes and sewage

Ridge-and-furrow irrigation for industrial waste disposal. F. H. Schraufnagel (*J. Wat. Pollut. Control Fed.*, 1962, **34,** 1117—1132).—A review is presented of this old method of wastewater disposal which could have modern applications, especially where the soil is suitable. The process requires little skill and is less expensive. The advantages and disadvantages of this method and the more recent spray irrigation method are discussed. (44 references.) B. F. FULLAN.

Toxicity to rainbow trout of spent still liquors from the distillation of coal. D. W. M. Herbert (*Ann. appl. Biol.*, 1962, **50**, 755—777).—The toxicity to rainbow trout of spent still liquors from the distillation of coal was due mainly to their content of NH_3 and monohydric phenols. An equation relating concn. of these with threshold concn. is presented. Based on these tests a method of predicting the likely toxicity of NH_3–phenol mixtures is described. Correspondence between predicted and observed toxicities of spent liquors was good enough for the method to be used for indicating the suitability of a liquor for discharge into a stream or for appropriate treatment before discharge. A. H. CORNFIELD.

Enterovirus removal by activated sludge treatment. W. N. Mack, J. R. Frey, B. G. Riegle and W. L. Mallmann (*J. Wat. Pollut. Control Fed.*, 1962, **34**, 1133—1139).—Two methods were used for testing for the presence of enteroviruses: (i) using monkey kidney epithelial cell cultures, (ii) using pad samples. The % of virus isolations from sewage decreased progressively through the plant. Plaque counts indicated that the amount of virus per unit vol. increased through the process. Chlorination produced a 92—95% reduction of virus particles in the final effluent. B. F. FULLAN.

Determination of the creatinine content of infiltration water. D. C. Abbott (*Analyst*, 1962, **87**, 494—497).—Standard solutions of creatinine from 0 to 10 ml. containing 10 μg. per ml. are diluted to 20 ml. and to each are added 1 ml. of $(NaPO_3)_6$ solution (10 g. each of $(NaPO_3)_6$ and NaCl in 100 ml.), 10 ml. of picric acid solution (1% w/v standardised by titration with 0·1N-NaOH to phenol red indicator) and 5 ml. of 0·5N-NaOH. After dilution to 50 ml. the extinctions are measured at 495 mμ to construct the standard graph. The sample is treated similarly. With coloured water another portion is treated similarly with omission of the picric acid. The difference in the extinctions is then a measure of the creatinine content. Recovery determinations with infiltration water, sewage and sewage effluents were satisfactory. A. O. JONES.

Analysis of air pollution mixtures: a study of biologically effective components. F. L. Estes (*Analyt. Chem.*, 1962, **34**, 998—1001).—Air pollution mixtures have been examined for oxidant-type components by the measurement of the inhibition in the growth of *Escherichia coli*. The oxidant material was separated by absorption in phosphate buffer and adsorption on a Na_2HPO_4-coated C-22 Firebrick column. The buffer completely removed unidentified material absorbing at 302 mμ, and it is suggested that this material contained a biologically effective agent which may be peroxyacetyl nitrate. R. A. HENDEY.

4.—APPARATUS AND UNCLASSIFIED

Spectrophotometric micro-estimation of some simple plant amines. M. Richardson (*Nature, Lond.*, 1963, **197**, 290—291).—A method for estimating amines in the presence of amino-acids has been devised by measuring the optical densities of the 2,4-dinitrophenyl deriv. formed on reaction with 1-fluoro-2, 4-dinitrobenzene. (17 references.) S. A. BROOKS.

Prediction of vapour–liquid equilibrium for polar–non-polar binary systems. R. N. Finch and M. van Winkle (*Amer. Inst. chem. Engrs J.*, 1962, **8**, 455—460).—Equations are developed, based on those of van Arkel, but modified by the inclusion of an asymmetry factor, for the prediction of the van Laar constants of binary mixtures of polar and non-polar compounds providing no hydrogen bonding occurs. The average deviation for 17 systems was 1·3 mol.%, but extension to ternary systems gave values in error by up to 5%. K. RIDGWAY.

Modification of the Monier-Williams (1930) method for [determining] sulphur dioxide. J. J. Thrasher (*J. Ass. off. agric. Chem., Wash.*, 1962, **45**, 905—907).—The procedure already proposed (*ibid.*, 1961, **44**, 479) has been further amended, and details of the method are given. The apparatus employed is precisely specified. Collaborative recoveries of SO_2 (from Na metabisulphite) ranged between 91·41 and 103·81% (average 97·23%). A. A. ELDRIDGE.

Sensitive qualitative test for sulphoxides on paper chromatograms. J. F. Thompson, W. N. Arnold and C. J. Morris (*Nature, Lond.*, 1963, **197**, 380—381).—A specific test is made by spraying the paper with an aq. solution of sol. starch, NaI and HCl. The paper is placed in a desiccator containing dry NaOH and after evacuation for 30—60 min. in the dark, sulphoxides appear as brown spots in ~15 min. S. A. BROOKS.

Paper chromatography of organic acids. H. A. W. Blundstone (*Nature, Lond.*, 1963, **197**, 377).—It is suggested that the best solvent for the separation of org. acids on paper chromatograms is n-butyl formate/formic acid/water (10 : 4 : 1). Bromophenol blue can be incorporated to give an automatically developed chromatogram. S. A. BROOKS.

Detection and quantitative estimation of synthetic organic pesticides by chromatography. R. T. Skrinde, J. W. Caskey and C. K. Gillespie (*J. Amer. Wat. Wks Ass.*, 1962, **54**, 1407—1923).—A general survey. Paper and gas chromatography are suitable for this determination but gas chromatography shows more promise and is able to separate large numbers of components and components of similar R_F. Analysis using argon and electron-capture ionisation detectors show that sensitivity of detection is increased with decrease in voltage in the detector cell. (45 references.) B. F. FULLAN.

Residue determination of 2,4-dichlorophenoxyacetic acid in dry crops and walnuts. A. Bevenue, G. Zweig and N. I. Nash (*J. Ass. off. agric. Chem., Wash.*, 1962, **45**, 990—993).—Yip's procedure (*ibid.*, 1962, **45**, 367) applied to crops containing a high % of solids (starch and proteins) gives low results. In the modified procedure described in detail the material is partially rehydrated before addition of the solvent for extraction. For samples fortified with 2,4-D before extraction, recoveries of 50—110% (average 85%) were obtained. A. A. ELDRIDGE.

Analysis of plant-protective agent residues. H. Frehse and H. Niessen (*Z. anal. Chem.*, 1962, **192**, 94—136).—A review. (224 references.) T. R. ANDREW.

Manual of fumigation for insect control. H. A. U. Monro (*F.A.O. Agric. Stud., Rome*, 1961 [1963], No. 56, 289 pp.).—The manual deals with fumigation for the control of insects above ground. It is written for the practical fumigator and may be of interest to senior planners and consultants in crop protection. Stress has been laid on the simpler and more readily available techniques. Principles underlying safe and effective fumigation are presented. Information has been collected from every part of the world especially that concerning fumigation of grain in bulk and citrus tree fumigation. (About 250 references.) E. M. J.

Recommended analytical methods for pesticides. X. Determination of suspensibility of water-dispersible powders. XI. Analysis for rotenone. Collaborative Pesticides Analytical Commee (*F.A.O. Plant Prod. Bull.*, 1962, **10**, No. 2/3, reprints, 3 pp. each).—X. A suspension of known concn. is prepared under standard conditions with standard hard water and placed in a prescribed measuring cylinder (250 ml.) at constant temp. After a specified time (30 or 10 or 30 and 10 min.) the top 9/10 is drawn off and the active ingredient determined in the remainder. Full details of the procedure are given. The method is not necessarily suitable for high-suspension concn. of a product as sometimes prescribed for use in aerial or low-vol. application.

XI. Rotenone is determined in ground lonchocarpus or derris roots and extractives by a polarimetric method applied to the rotenone which is separated as its CCl_4 complex. The root is extracted with $CHCl_3$ and the rotenone transferred to benzene–ether (**I**) solution which is washed with 2% KOH to remove impurities. Resin or rotenone for assay is dissolved in **I** and then washed with 2% KOH. The rotenone is finally taken up in CCl_4. Full details are given. H. S. R.

Isolation and identification of flavonoid compounds in cottonseed by paper chromatography. C. Pratt (*Dissert. Abstr.*, 1962, **23**, 811—812).—The compounds were identified by chromatographic comparison of the isolated compounds and their hydrolysis products with known standards; u.v. absorption spectra of the compounds and their degradation products; shifts of absorption max. in the u.v. range on treatment with certain chemicals; and quant. analysis of the ratio of sugar to aglycone. Compounds isolated were kaempferol-3 rhamnoglucoside, quercetin-3-gluco-glucoside, rutin and isoquercetin. Two other compounds are quercetin deriv. J.A.C. ABSTR.

Clams as indicators of strontium-90. D. J. Nelson (*Science*, 1962, **137**, 38—39).—Clam shells from the Tennessee River system have been analysed for their ^{90}Sr content. Samples were taken from various points near the effluent from Oak Ridge National Laboratory. The ^{90}Sr remained in solution and the concn. at any distance up to 500 miles from the effluent outfall could be predicted on dilution data. T. G. MORRIS.

Strontium-90 in Alaska. A. R. Schulert (*Science*, 1962, **136**, 146—147).—In general the foodstuffs consumed by the bulk of the Eskimo population have ^{90}Sr content similar to that of other foods in the north temperate zone. Caribou, which feed on the mosses and low vegetation overlying the permafrost zone, have 10 to 20 times the ^{90}Sr burden of that of domestic cattle. Eskimo for whom caribou is the staple diet have been found to have four times the average ^{90}Sr content. T. G. MORRIS.

Strontium-90 in man. J. L. Kulp and A. R. Schulert (*Science*, 1962, **136**, 619—632).—A review. T. G. MORRIS.

Journal of Applied Chemistry

The following papers are appearing in the July, 1963, issue

Sucrose acetoacetates
By L. K. Dalton

An *X*-ray examination of internal corrosion products from power station boilers
By E. Bullen and J. W. Jeffrey

Adsorption of organo-clay derivatives
By G. B. Street and D. White

Spontaneous combustion of hay
By H. P. Rothbaum

Quantitative study of the oxidative discoloration of ethyl linoleate. I. Oxidation in the bulk phase
By F. Franks and B. Roberts

Inert diluents for use in nuclear energy plants. III. Comparison of the stability of some conjunct polymers of simple olefins with odourless kerosene
By E. S. Lane and M. J. Holdoway

Infra-red study of hydrothermal conversion of alumina trihydrate into alumina monohydrate
By Taichi Sato

Silica powders of respirable size. III. Dialysis of quartz powders against dilute sodium hydroxide
By I. Bergman

Selective reduction of gibberellic acid
By D. F. Jones and P. McCloskey

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CONTENTS

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