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PHOTOMETRIC METHODS IN BACTERIOLOGY*

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The elementary features of light-scattering by small particles are described and illustrated by examples relevant to bacteriological practice. A number of specialised applications are reviewed.

Introduction

THE uses of photometric measurements in bacteriology are of two kinds: (i) for the determination of some optical property of the substance of the organisms in a suspension, e.g., the mean refractive index or the absorption coefficient at some particular wavelength—these are of somewhat specialised interest; (ii) for estimating quickly and conveniently size and concentration of organisms—of wide practical application. Measurements of the first kind are rendered more difficult by the particulate nature of the material, but those of the second depend directly on the fact that small particles scatter light incident upon them even if they do not absorb it.

Since the vessels generally used to contain bacteria are very large compared with the organisms themselves, there is a temptation to treat a suspension as sensibly continuous like a true solution and to suppose that it obeys Beer's law. If this were the whole truth, or even most of it, the photometry of suspensions would be much simpler than it is. The difficulties which arise stem from the size of the organisms, which is of the same order of magnitude as the wavelength of visible light. The interaction with light of particles of this size cannot be treated by the methods of geometrical optics; for instance, they cast no definite shadow.

Scattering of light by particles

The scatter from even a spherical, optically homogeneous particle is quite complex. Fig. 1(a) is typical, but the detailed shape of the polar scattering diagram varies rapidly with the ratio of wavelength to radius. In a suspension of bacteria the particles are not homogeneous (as shown by the structure visible under the phase microscope), they are not usually spheres, and they have an appreciable range of size. Moreover the light scattered in a given direction from a non-spherical particle varies in intensity (and polarisation) with its attitude relative to the incident light. The result is a kind of average in which the maxima and minima are smoothed out: the scattered intensity round a small bulk of the suspension is as shown in Fig. 1(b)—most of the light removed from the incident beam is deflected through only a small angle. The strong forward scattering is made quite obvious by examining at different angles a suspension through which a narrow beam of light is passed. The general shape of the diagram is the same over a wide range of wavelengths, but (i) the total scattered light is less, the smaller the ratio of particle size to wavelength; (ii) the smaller the ratio of wavelength to particle size, the more exaggerated is the forward scatter; (iii) the intensity of the scattered light is greater, the greater the difference between the refractive indices of particles and suspending medium. The working of rule (i) is seen in the reddening of white light passed through a suspension, and

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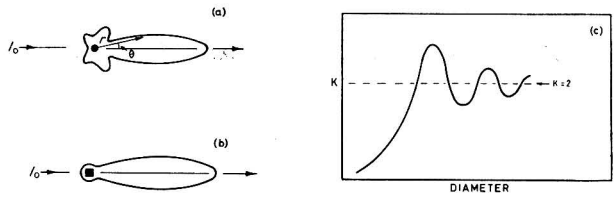

FIG. 1.—(a) *Polar scattering diagram of a small spherical particle of radius comparable with the wavelength* I_0: incident beam; the length of the radius vector r is proportional to the intensity of scattering in the direction θ (schematic)

(b) *Polar scattering diagram for a suspension; average effect produced by dispersion of size, shape and refractive index* (schematic)

(c) *Total scattering coefficient, K, of transparent spherical particles as function of their size* (schematic)

of rule (iii) in the different visibilities of thin layers of organisms on agar plates: compare for instance *Bacillus anthracis* with *Proteus vulgaris*; on the whole the optical density of the cell wall is the greater in Gram-positive organisms.

Effect of ratio of wavelength to particle size on forward scatter

Rule (ii) is illustrated in the following example. An instrument for absolute turbidimetric measurements[1] was used to measure the light scattered at various angles from suspensions of three different organisms. The suspending medium was tryptic meat broth, and the illuminant mercury green light of wavelength 546 mμ. In a suspension of *Chromobacterium prodigiosum* (resting cells), the relative intensities of scattering S in directions making angles θ with the illuminating beam were as follows:

θ	30°	40°	50°	70°	90°	110°	130°
S	1093	517	257	84·8	40·3	33·0	39·5

Similar measurements were made on *Bacillus subtilis* and *B. megatherium* (vegetative organisms). The ratios of the scattered intensities at 30° and 110° from the illuminating beam were compared:

	S_{30}/S_{110}	Mean size of organisms, μ
Chr. prodigiosum	33	1·7 × 0·5
B. subtilis	60	3·6 × 0·6
B. megatherium	92	7·6 × 1·2

The results confirm rule (ii) since the intensity ratio increases with size of organism, and they suggest how scattering measurements could be used to compare the sizes. There would of course be complications if the organisms were not of similar shape.

Attenuation of light by suspension

Consider now the attenuation of a beam of light incident normally on a plane layer of a suspension:

$$I = I_0 \exp(-kcx)$$

where I_0 is the incident and I the transmitted intensity, x is the thickness of the layer, c is the concentration in number of particles per unit volume, and k is a constant, the 'specific extinction coefficient', depending on the particle sizes and geometry and on the refractive indices of particles and solution. The equation can also be written in the form

$$I = I_0 \exp(-KAcx)$$

where A is the mean projected area of the particles in the direction of the illuminating beam and K is a constant (for suspensions of transparent spheres all of the same size, K is called the total scattering coefficient). Now if the laws of geometrical optics held we should expect the particles to divert from the illuminating beam just as much light as they intercept and to find $K = 1$. But in fact K is a very complex function of size even for suspensions of uniform spheres (Mie.[2] See Fig. 1(c)). A point of special interest is that for particles (of no matter what shape)

very large compared with the wavelength, K tends to the limiting value 2, i.e., a large particle removes just twice as much light as would be expected from the area of its profile. Although it has been known for a long time, this fact has not found ready acceptance everywhere because of seeming contradictions in everyday experience. As late as 1946, Rose & Lloyd[3] expressed surprise at their experimental finding that K could be greater than 1. The reason for the difficulty is that any detector of light has a finite angular aperture, and so will collect some of the scattered light even if the illuminating beam is almost perfectly parallel; it will collect relatively more the larger the particles because, for large particles, half of the scattered light is deflected only very slightly from the direction of the incident light. Rose & Lloyd obtained their result by restricting the aperture of their detector severely by means of stops. Only quite recently, a refinement of Rose & Lloyd's procedure has enabled the theory to be quantitatively verified (e.g., Lewis & Lothian;[4] Bateman, Weneck & Eshler[5]).

When a layer of suspension has an optical density less than about 0·1, the individual particles can be treated as scattering independently of one another.[6] At higher optical densities secondary scattering becomes important, that is, an appreciable amount of light emerging from the suspension has encountered more than one particle, and Beer's law begins to fail for this reason. Ordinary absorptiometers may seem to follow the law up to a higher density, but the indicated values are not meaningful, being so greatly dependent on the structure of the instrument. The following experiment illustrates this dependence.

Dependence of apparent optical density on type of absorptiometer

Measurements of apparent optical density were made on suspensions of *Bacterium aerogenes* in normal saline. The mean length of the organisms was 5·4μ, the mean breadth 0·86μ. Suspensions were contained in optically worked cells 1 cm. thick, and green light was used. Two instruments were compared:

(i) the well-known 'Spekker' absorptiometer;
(ii) an experimental instrument with collimated source and restricted acceptance angle at the detector (Fig. 2).

The results are shown in Table I.

Users of the 'Spekker' will know from its construction that light scattered at appreciable angles to the primary beam can still reach the detector photo-cell; the above figures show how inclusion of some scattered light with the transmitted light diminishes the apparent optical density.

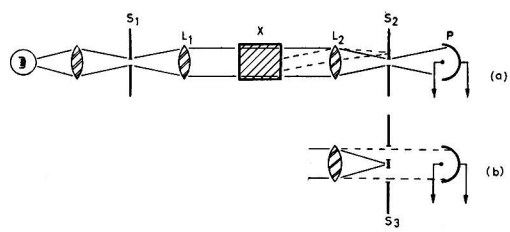

FIG. 2.—(a) *Experimental photometer with restricted acceptance angle at the detector*

Light from a small illuminated stop S_1 passes as a parallel beam through the specimen X, and is focused on a similar stop at S_2. Since S_2 is in the focal plane of the lens L_2, points of S_2 correspond to directions in the space between L_1 and L_2. Light scattered at quite small angles to the illuminating beam falls outside the hole in S_2 and is not received by the detector P

(b) *Collection of light scattered at small angles*

(b) In the wide-range nephelometer, the stop S_2 (Fig. 2a) can be alternated with an annular stop S_3 which blocks the direct light but permits the photocell to receive light scattered near to the primary beam. The scattered and transmitted intensities can thus be compared

Of course the error hardly detracts from the value of the 'Spekker' as a convenient instrument for comparing the density of suspensions.

The complexity of the interaction between light and suspended particles has given rise to many contradictory statements in the literature. Thus Burns[7] found that in his experiments the optical density was proportional to the concentration of cell protein, irrespective of the size

Table I

Apparent optical density of suspensions measured by two types of instrument

Concentration of organisms, no. per c.c. × 10^{-9}	Apparent optical density 'Spekker'	Restricted aperture
2·6	1·44	2·42
0·43	0·36	0·97
0·072	0·063	0·15
0·024	0·019	0·039

of the organisms. He supported with references a conclusion that turbidity measurements estimate particle concentration by mass, not by number. There is no reason to doubt the truth of such statements in their immediate context, but they are not general truths about light scattering. Conclusions to the opposite effect are perhaps equally common. There is an interesting example in a paper by Stárka & Koza.[8] They followed the synchronous growth of bacteria with a standard Pulfrich nephelometer, in which the scattered light is collected over a fairly wide range of angles about 45° away from the illuminating beam. They found by comparison with direct counts that, during the periods of growth without fission, the scattered light remained constant, and during the periods of high fission rate it increased steeply; that is to say, the turbidity increased in the same stepwise manner as the concentration of organisms by number. Powell & Stoward[9] repeated and confirmed these observations with a similar instrument, but Abbo & Pardee,[10] with a different form of nephelometer, obtained much less clearly marked steps. The reason for this behaviour seems to be that as the organisms increase in size, more light is in fact scattered, but a greater proportion is thrown forward, near to the illuminating beam; at angles near 45°, the intensity is not much altered.

All these considerations show the difficulty of assimilating to one another measurements made on different suspensions and with different instruments. Brown's tubes represent perhaps the simplest application of photometry in bacteriology. Their originator well understood their limitations (Brown & Kirwan[11]); his followers have not always been as critical. The following experiment demonstrates clearly the necessity of calibrating each combination of instrument and suspension.

Suspensions of *Chr. prodigiosum* and *B. megatherium* in normal saline, each at four concentration levels, were compared on two instruments:

(i) the 'Spekker' absorptiometer; (ii) the 'EEL' nephelometer.

In the 'EEL' instrument, the sample is contained in a test-tube along whose axis a narrow beam of light is directed from below. The scattered light is received on a bank of photoelectric cells surrounding the tube. Thus the cells can receive only light scattered in the angular range 45–135° to the beam, since 45° (about) is the critical angle for a glass–air–surface—the rest of the scattered light emerges at the ends of the tube.

The results are shown in Fig. 3 from which it is seen that the curves for the two organisms differ considerably. Qualitatively, the difference is easy to understand. *Chr. prodigiosum*, being the smaller organism, scatters relatively more light at large angles to the illuminating beam; of two suspensions giving the same apparent density on the 'Spekker', the one having the smaller particles will give the larger reading on the 'EEL'. It is especially noteworthy that two suspensions of comparable concentration, such as are represented by X and Y (~5 × 10^8/c.c.) in Fig. 3, will give readings on the 'EEL' suggesting that the turbidities are in the order X > Y, while the 'Spekker' readings suggest the opposite: Y > X.

Use of the physical principles of light scattering

A review is now made of some special investigations and instruments in which the physical principles of light scattering have been exploited.

(A) *The International Opacity Standard*

The complex of factors on which turbidimetric measurements depend is well illustrated by Spaun's recent studies[12] on the International Opacity Reference Preparation.

As supplies of the original preparation became scarce, Spaun attempted to copy it by

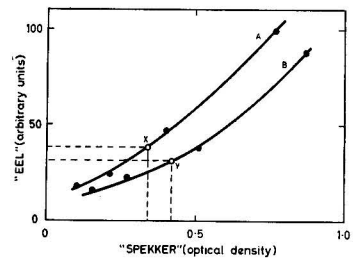

FIG. 3.—*Comparison of* Chr. prodigiosum *(curve A) and* B. megatherium *(Curve B) suspensions on the 'EEL' and 'Spekker' instruments*

Solid circles are experimental points for different concentrations. Suspensions of comparable concentration such as X, Y have apparent turbidities in the order X > Y or Y > X according to the instrument used

suspensions of glass particles in water. His investigation showed how much the readings of various instruments could differ from one another, and his great problem was to make up a suspension which should have the same apparent opacity as the original when measured in several different instruments in common use. He was able to achieve his end only by making up trial mixtures of suspensions ground to different degrees of fineness, so that the scattering pattern of the original standard was reproduced in some detail.

Spaun further showed the lack of correspondence between opacity and concentration in a variety of suspensions of bacteria. In particular, he found that for a given opacity, formalin-killed vaccines had only about 2/3 the dry mass concentration of heat-killed vaccines of the same organisms. This result can be understood by reference to the appearance of the organisms in the phase-contrast microscope: the formalin-killed organisms gradually lose their contrast as the cell contents are leached out, but the cell walls remain almost unchanged in contrast and dimensions. It does not follow that the formalin-killed organisms are less effective immunologically, on the contrary, in this case the opacity is probably a better measure of antigen content than is the mass concentration.

A concomitant effect of formalin treatment is to increase the electrical conductivity of the organisms, a circumstance which explains such anomalous results as those of Lark & Lark.[13]

(B) *Spore germination*

The dependence of turbidity on refractive index can be made the basis of a method for detecting and measuring the germination of spores in bulk. That the refractive index of spores falls during germination is obvious from observations with the phase microscope.[14] The refractive index of both germinated and ungerminated spores is greater than that of normal aqueous media, and since germination is not accompanied by an appreciable immediate change in size, its occurrence in a suspension produces a fall in turbidity. The effect is often so marked as to be visible to the naked eye.[15] No special instrument is required to follow the change and to make comparative measurements. An example is given by Powell,[16] who established a quantitative correspondence between turbidity and the proportion of germinated spores in a suspension.

(C) *Wide range nephelometer*

The intensity (T) of the light transmitted by a suspension is approximately proportional to the quantity $\exp(-kxc)$, where k is a constant, x is the thickness of the suspension in the direction of the light, and c is the concentration by number of particles. If the suspension is not too dense, it is evident that the light (S) scattered in any direction close to the primary beam is jointly proportional to c and $\exp(-kxc)$, approximately. For in these circumstances the effects of the separate particles are additive and the light which is scattered by any one of them has to pass through a total depth of suspension only just greater than x, the thickness of the suspension in the direction of the primary beam; this is true whatever the position of the particle within the illuminated volume (Fig. 4a). We thus have

$$\frac{S}{T} \propto \frac{c\exp(-kxc)}{\exp(-kxc)} = c$$

A nephelometer was constructed on this principle by Powell[17] (see Fig. 2b). It was found to possess the above property of linearity over a range of concentration of several hundredfold; the total useful range was about $10^4 : 1$. This is a great advantage in handling suspensions of pathogens, which do not usually need to be diluted before measurement; because of the intense forward scatter, the photoelectric cell is not required to be especially sensitive, and the range of intensity which it is required to accept is less than the range of concentration covered. The disadvantages are the relative complexity of construction and the susceptibility to interference by dust and bubbles and large foreign particles in the suspension.

A similar principle was used by Briggs[18] in an instrument of more sophisticated design. By means of a system of mirrors the light from a lamp was divided and made to impinge on a suspension from two directions at right angles. A photoelectric cell adjacent to the containing vessel received both transmitted light and light scattered at 90° to one of the illuminating beams. The two beams were chopped at different frequencies and the corresponding a.c. outputs from the photocell separated and amplified electronically. The two outputs were compared in a bridge, and finally their ratio was delivered to a continuous recorder. Readings were not affected by factors which interfered similarly with the two light beams, e.g., change in lamp output, colour of suspending fluid, deposits on the walls of the containing vessel. Range and linearity were somewhat less than in Powell's instrument,[17] but the stability of the system over long periods was assured by the use of a.c. amplification.

It is likely that an instrument of this kind will prove to be of great value for the monitoring and automatic control of continuous cultures.

(D) *The true light absorption of bacteria*

The difficulty of measuring the absorption spectra of substances contained within bacteria (and indeed of small particles generally) is well known. It has often been found that the absorption spectra of bacterial suspensions do not correspond with those of substances known to be contained within the organisms; this has given rise to unjustified suggestions that the absorbing substances are chemically associated in some way which alters their spectra. Attempts have been made by statistical methods to correct the absorption spectra of suspensions so as to obtain the true absorption of the substance of the particles (e.g., Duysens[19]). Such methods have their place for larger organisms, but for bacteria they are not realistic—the root of the difficulty is again the failure of geometrical optics.

The experiment of placing a small opaque disc between the eye and an illuminated pinhole at some distance away will be familiar to many; the pinhole can still be seen when the axis of the disc coincides with the line joining the pinhole to the eye; in fact the shadow of the disc has a bright spot of light at its centre. A disc of red glass, illuminated with a mixture of red and green light, can give a predominantly red shadow with a bright green central spot; that is, the bright spot is of the colour of the light absorbed by the glass. The same phenomenon occurs on the smaller scale of a bacterial suspension in a photometer, and it is quite possible for the geometry of the system to be such that an absorption band is suppressed or even reversed, appearing as a spectral region of high transmission. An example of reversal is described by Lothian & Lewis.[20]

It is evident that if all the light, both scattered and transmitted by a suspension, could be collected and compared with the incident light, any defect discovered would be due to true absorption. An imperfect approach to this ideal consists in placing a diffusing screen immediately behind the vessel containing the suspension,[21] but a most elegant solution to the problem has been devised by Bateman & Monk.[22]

It is known that if a source of light is placed anywhere within a sphere having a uniform diffusely reflecting inner wall (an ' integrating sphere '), the light intensity at the wall is everywhere the same and for a given total light output is independent of the radial distribution of the source. Bateman & Monk therefore placed their suspensions in a small quartz flask embedded in magnesium oxide which acted as a diffuse reflector. They illuminated it through the neck and observed the diffused light through a small hole in the mantle. The diffused light behaved as if it had travelled through a great depth of suspension without attenuation by scattering, and its spectrum corresponded closely to the true absorption of the particles.

Bateman & Richetta have since developed a more sophisticated instrument on these lines, but no details have yet been published.

(E) *The length of rod-like organisms*

The shape and size of a particle can in principle be inferred from its light-scattering properties, and as the first example above suggests, it is possible to infer at least mean values from the scattering of bulk suspensions. A rather complicated set of measurements is required to enable this to be done even in ideal cases, while a suspension of bacteria with its particles of unequal size, optically inhomogeneous and of a geometrically awkward shape, is not amenable to exact theoretical treatment. There is nevertheless a need for a simple and rapid means of following changes in the length or axial ratio of rod-shaped organisms, particularly in the study of synchrony and of growth in continuous culture.

When light is incident on a long thin rod, the scattered light is concentrated in a set of preferred directions, namely the cone whose axis coincides with that of the rod, and one of whose generators is the direction of the incident beam (Stadie,[23] see Fig. 4*b*). (The existence of a preferred direction is well exemplified by a long single filament of spider's web against a dark background in sunlight. It often happens that the line of sight to a part of the filament lies in the preferred cone, and that part is then seen as a bright thread even when the web is much too far away to be resolved by the eye.) When the scattering rod is normal to the beam, the cone opens out to a flat sheet normal to the rod and containing the direction of the incident beam. The behaviour of short rods and ellipsoids of small axial ratio is qualitatively similar to that of fine filaments, and the degree to which the scattered light is concentrated in the preferred directions is a measure of the length. Hence if we have a means of aligning the organisms in a suspension so that their long axes ars normal to the direction of an illuminating beam and to a convenient direction of observation, the intensity of the scattered light received will be greater than if the organisms are disposed at random. The excess will be a measure of the length. Partial but sufficient alignment is readily achieved by causing a suspension to flow through a tube without turbulence ; except on the axis of the tube, where there is no shear, the organisms tend to set themselves parallel to it.

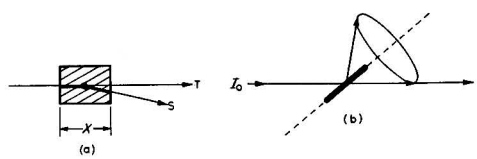

FIG. 4.—(a) *Principle of wide-range nephelometer*

Light (*T*) transmitted by a suspension is attenuated by a factor $\exp(-kxc)$; light (S) scattered near to the incident beam has to pass through approximately the same depth x of suspension (thickened line) and so its intensity is proportional to both c and $\exp(-kxc)$

(b) *Scatter by a thin rod*

The scattered intensity is highest on a cone whose axis is that of the rod and which contains the direction of the incident beam (I_0)

Powell & Stoward[9] made a very simple modification to a standard Pulfrich nephelometer to enable it to be used in this way. In this instrument as normally used, the sample container is a cylindrical glass cell illuminated transversely. Light scattered near 45° to the beam is compared visually with the lamp. The cell was replaced by a tube 5 mm. in diameter, fitted with a tap, and, to permit measurements to be made rapidly, a pair of photoelectric cells was substituted for the comparator eyepiece. When the suspension was allowed to run out, the increased intensity of the scattered light during flow was determined electrically.

This instrument has been used successfully for following the synchronous growth of bacteria, to detect the periods of change in mean length consequent upon fission. The increase in scattered intensity during flow can be surprisingly large. For *Bacterium aerogenes* with an axial ratio of 7 : 1 the change is about 60%, and even for *Bacillus subtilis* spores with a ratio of 1·8 : 1 it is about 3%.

Acknowledgment

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SOME VOLATILE COMPOUNDS FROM COOKED POTATOES

By R. SELF,* H. L. J. ROLLEY† and A. E. JOYCE‡

An investigation of some of the volatile compounds produced by boiling potatoes has been carried out by gas chromatography. The method used for the concentration, separation and identification of small samples of such compounds is described. These were shown to include hydrogen sulphide, acetaldehyde, methanethiol, acrolein, acetone, ethanethiol, dimethyl sulphide, iso- and n-butyraldehyde, isovaleraldehyde and methyl isopropyl ketone, along with some unidentified components. These findings are discussed in relation to the Strecker degradation of amino-acids, and in the light of present knowledge of flavour components.

Introduction

During the development of accelerated-freeze-dried foodstuffs,[1] it was soon found that it was essential to start with a fully flavoured raw material in order to obtain a well-flavoured product. Small undesirable changes in flavour soon became apparent during the pretreatment and drying, even when every precaution was taken. Investigation of the suitability of different varieties of fruits and vegetables for accelerated-freeze-drying showed that there was a lack of information on the nature of the organic constituents of the tissues, and especially the more volatile compounds. These compounds are probably responsible for the more delicate and

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highly prized qualities of flavour which are very difficult to measure by organoleptic techniques.[2] There is also some suggestion that certain more recently introduced varieties of fruits and vegetables which are higher in yield and have other desirable attributes such as resistance to disease, are weaker in flavour. This may be due to the fact that until recently, plant breeders had not any methods for measuring or investigating flavour.

In devising a programme of research on flavour, potatoes were chosen because they tended to show the type of flavour losses described above and because the raw material was available for most of the year. This paper contains the results of a study designed to identify the more volatile components of the flavour of cooked potatoes.

Experimental

Raw material

Throughout the greater part of this study the variety King Edward was used. These were supplied through the kindness of Lord Glentanar from the Home Farm, Glen Tanar, Aboyne, Aberdeenshire. They were grown on land which had been limed on the basis of soil tests made by the Macaulay Soil Research Institute. Dressings of farmyard manure had been applied annually in rotation together with home-made compost, bone meal, steamed bone flour, herring guano, ground mineral phosphate and potassic mineral phosphate. The land had also been grazed by cattle, sheep and poultry on free range. The potatoes were exceptionally free from blemishes and had good keeping qualities. They were stored in 1-cwt. sacks in a store conditioned at 10° and thus insulated from possible frost damage.

Preliminary investigation on production of volatile compounds

(a) *Production from raw tuber.*—Air passed through activated charcoal was slowly drawn over 2 kg. of whole, washed, soil-free potatoes and then through a series of cold traps (solid carbon dioxide/acetone), for 3 months. The traps were changed at weekly intervals and examined for smell. Freshly cut potato tissue was also examined for smell, both in the absence of and presence of sodium fluoride.

(b) *Production from cooked tuber.*—In a circuit similar to that used for the raw potatoes it was found that the aroma of cooked potatoes did not develop strongly until the temperature of the cooking liquor was over 90°.

Apparatus for gas chromatography

The columns used were 50-, 100- and 200-ft. lengths of 0·01 in. i.d. 'Maranyl' nylon capillary, described by Golay,[3, 4] the length varying according to the separation required. Columns 0·02 in. i.d. were used for the larger samples necessary in the identification of separated components by their smell. The columns were coated with liquid phase under conditions similar to those described by Scott,[5] the difference being that 200 cm. of the columns were filled with a 15% v/v solution of the phase in ether instead of the suggested 20 cm. with 10% v/v. The two phases used were 'Embaphase' silicone oil, and 'Embaphase' tritolyl phosphate (T.T.P.). Film thicknesses (df) varied from 4 to 5 $\times$ 10^{-5} cm. These columns were operated at an optimum practical gas velocity (O.P.G.V.) of 17 cm. $sec.^{-1}$, with a 30 : 1 gas stream splitter. The Lovelock[6] small detector was used with an 0·5 curie tritium source, operated at either 1500 or 1800 V, in conjunction with the power pack and amplifier described by Scott.[5] For mixed chromatography, however, the separation was improved by reducing the gas velocity to 8 cm./sec. (O.G.V.).

Sampling of volatile substances from potatoes

It was essential to have the sample for examination as dry as possible when using the argon detector and a stationary phase which was not specifically intended for use with water vapour, otherwise areas of the chromatogram were 'quenched'. In the work on the volatile constituents of coffee[7] and meat[8] use was made of an inert gas stream, cold traps and vacuum techniques, both for removing volatile matter from the material under investigation and for its separation from water vapour. The inert gas stream technique showed that it was very

difficult to obtain an inert gas sufficiently free from traces of impurities, which caused interference with the chromatogram, at the flow rate required. The chromatographic analysis of samples of air from the laboratory showed that a vacuum system would need to be absolutely leakproof, or similar interference would take place.

It was decided to use a system in which the volatile compounds were allowed to accumulate at atmospheric pressure and an elevated temperature. The apparatus consisted of a wide-necked 2-litre reaction vessel with a ground-glass head into which fitted a 12-in. condenser with a large condensing area in relation to its internal volume. The end of the condenser was closed by a rubber bung pierced by a hypodermic syringe needle. One kg. of washed unpeeled King Edward potato slices were placed in the vessel and covered with double glass-distilled water and boiled. After 30 min. the samples of volatile products were withdrawn slowly (5–10 sec.) via the needle in the bung into a 5- or 10-ml. ground-glass syringe. When the sample was removed slowly, the reflux condenser retained sufficient of the water to prevent blocking the capillary trap and desensitising the detector.

This sampling system is not quantitative, because volatile compounds, particularly those of higher boiling point, were adsorbed on to the surface of the syringe and fittings. It was necessary, therefore, to arrange a nylon capillary trap immersed in liquid oxygen between the sample source and the syringe. For semi-quantitative work the barrel of the syringe was withdrawn by a motor-operated screw geared down to the required speed (2 ml. per 5 min.). This enabled a sample to be taken without loss of the very volatile fractions.

Concentration and injection of volatile matter

The enrichment trap technique[9] was modified from experience gained in mixed chromatography to give a technique which produced better resolution for samples of potato. Volatile compounds were collected in a nylon capillary enrichment trap[9] which was then connected in series to a second trap only half-immersed in liquid oxygen. An all-glass 1-ml. syringe containing air free from volatile compounds was connected to the free limb of the first enrichment trap, which was then removed from the liquid oxygen and immersed in hot water (60–70°) for 10 sec. After removal from the hot water, 0·2 ml. of air (approximately four times the volume of the trap) was slowly injected into the system over a period of 1 min., to transfer the volatile compounds to the second trap, where they were recondensed in a much smaller volume (approximately 0·002 ml.) in the inlet limb.

Once the transfer was complete, the second trap was immersed deeper in the liquid oxygen, to prevent any back-diffusion of the volatile compounds, and disconnected from the enrichment trap. The volatile compounds were injected on to the column by placing this second trap, after connexion, into hot water (60–70°) for 10 sec. When an authentic sample was required with the unknown for mixed chromatography, it was injected from a syringe into the enrichment trap, still immersed in liquid oxygen, before the transfer to the second trap had taken place.

Preliminary model experiments with amino-acid decomposition

Equimolecular quantities of cysteine, cystine and methionine and dehydroascorbic acid[10] were mixed in turn at a concentration of 0·005 molar at pH 7 and heated at 90°. The products of decomposition were examined by absorption in mercuric cyanide (4% w/v) and mercuric chloride (3% w/v)[11] and 2,4-dinitrophenylhydrazine (0·2% w/v in 2N-HCl).[12] Commercial nitrogen purified by passing through mercuric chloride was used as a carrier gas, and also air was treated in the same way. The mercuric chloride tube was exchanged with a tube of saturated chloramine T[13] during the experiment. Separate portions of the precipitate from the mercuric chloride tube were decomposed with 10% sodium hydroxide and 6N-hydrochloric acid.[14] The vapours from the alkali decomposition were aspirated separately through either mercuric chloride (3% w/v) or saturated aqueous chloramine T. Those from the acid decomposition were aspirated through distilled water (to remove hydrochloric acid vapour), then either into mercuric cyanide (4% w/v) or sodium hydroxide solution in ethanol (5% w/v) or lead acetate solution (20% w/v). The ethanolic sodium hydroxide solution after aspiration

was added to cold saturated 1-chloro-2,4-dinitrobenzene[14] in alcohol. The chloramine T solutions were examined by paper chromatography for sulphidimines.[13]

Results

(*a*) From the preliminary investigation on the production of volatile compounds, it was found that whole potatoes, over a 3-month period, emitted only an aroma best described as ' earthy '. The smell obtained from fresh cut potato was different from that of whole or cooked potato, and was not inhibited by sodium fluoride but was removed by washing the cut surface.

(*b*) The chromatogram (Fig. 1) was obtained by selective collection during 10 h. boiling. It was impossible, however, to run a large sample to show the trace components without contaminating the detector with excess hydrogen sulphide, which resulted in poor response. Such components were therefore identified in a fraction obtained by passing a 5-ml. gas sample rapidly through the enrichment trap. This technique allowed the greater part of the hydrogen sulphide to be ' flushed ' straight through. Although a graded loss of all other components, including the higher-boiling compounds, was incurred, most of the minor components could be detected without too much difficulty.

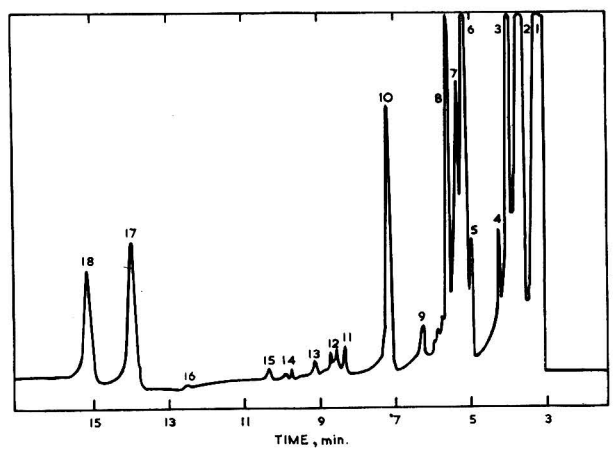

FIG. 1.—*The low-boiling volatile compounds from potatoes*

Gas chromatography on 100 ft. of 0·01-in. diameter nylon capillary packed with silicone oil (Full scale deflection = 10)

Identification of labelled peaks	Confirmation	Identification of labelled peaks	Confirmation
(1) Hydrogen sulphide	mixed chromatography and odour	(10) Isobutyraldehyde	mixed chromatography and odour
(2) Acetaldehyde	,, ,, ,, ,,	(11) Unknown	
(3) Methanethiol	,, ,, ,, ,,	(12) Unknown	
(4) Unknown		(13) n-Butyraldehyde	tentative-retention times
(5) Acrolein	tentative-retention times	(14) Unknown	
(6) Acetone	mixed chromatography	(15) Unknown	
(7) Ethanethiol	mixed chromatography and odour	(16) Unknown	
(8) Unknown		(17) Isovaleraldehyde	mixed chromatography and odour
(9) Dimethyl sulphide	mixed chromatography and odour	(18) Methyl isopropyl ketone	,, ,, ,, ,,

Different stationary phases (Table I) were used to improve the resolution of the chromatogram and provided confirmatory evidence of the identity of the various components present. Further confirmation was obtained from mixed chromatography.

The relative amounts of high- and low-boiling components in the sample are likely to be different from those generated in the disintegrating cells of the potato tissue, due to the need to remove water from the vapours of the cooking potato. Such differences will be due, not only to the temperature gradient in the condenser, but also to adsorption on surfaces and the ratio of the internal volumes and the surface area of the condenser and boiling flask. The

other drawback of the system is that the alcohols were completely absorbed on the nylon columns. As the volatile products evolved from the boiling potatoes increased in concentration, the volume of vapour necessary to obtain a satisfactory chromatogram could be reduced to 1 ml. When handling these relatively small samples of vapour from the cooking vessel, for examination by this capillary column system, the greatest care had to be taken to exclude impurities arising from the laboratory atmosphere.

Table I

Comparison of potato volatile constituents on three stationary phases on 50 ft. of 0·01-in. diameter nylon capillary

Volatile compound	Boiling point, °c	Silicone oil Relative retention time	Tritolyl phosphate Relative retention time	Dinonyl phthalate Relative retention time
Hydrogen sulphide	−61·8	0·13	0·07	0·11
Acetaldehyde	21	0·31	0·30	0·33
Methanethiol	6	0·47	0·23	0·29
Acetone	56	1·0	1·0	1·0
Ethanethiol	36	1·24	0·86	0·69
Dimethyl sulphide	36	1·33	0·58	0·80
Isobutyraldehyde	64	1·93	1·30	1·15
Isovaleraldehyde	92	5·15	3·81	5·08
Methyl isopropyl ketone	94	5·67	3·81	5·16

(*c*) From the model experiments with amino-acid decomposition it was found that with cysteine and cystine and air aspiration, black precipitates were obtained with mercuric cyanide, orange precipitates with 2,4-dinitrophenylhydrazine, and no precipitates with mercuric chloride. The black precipitate from the mercuric cyanide was subjected to acid decomposition[14] and aspiration into mercuric cyanide and ethanolic caustic soda. No precipitates were obtained from the former, or from the latter when added to 1-chloro-2,4-dinitrobenzene. The 2,4-dinitrophenylhydrazine precipitate was not further examined.

Under the same conditions, methionine and dehydroascorbic acid with air gave precipitates with mercuric cyanide, mercuric chloride and 2,4-dinitrophenylhydrazine solutions. The mercuric cyanide precipitate when boiled with water and recrystallised from ethanol had m.p. 178°, unchanged by further recrystallisation. The mixed melting point with authentic mercury methanethiol of m.p. 178° was 178°. A portion of this mercury compound, on decomposition by 6N-hydrochloric acid and aspiration into ethanolic caustic soda followed by reaction with 1-chloro-2,4-dinitrobenzene, gave pale yellow crystals of m.p. 125°. These were recrystallised from ethanol to give m.p. 127°. The mixed melting point with authentic 2,4-dinitrophenyl methyl sulphide of m.p. 128° was 127–128°.

The mercuric chloride precipitate required at least six collecting tubes before a tube free of precipitate could be obtained. This slow reaction was taken as indicating the presence of a disulphide[14] as well as possibly of a sulphide. A portion of this mercury compound after acid decomposition gave a mercuric cyanide compound, also a 2,4-dinitrophenyl sulphide and lead salt. The mercury compound was boiled with water and recrystallised twice from ethanol; it melted at 178°. A mixed melting point with authentic mercury methanethiol of m.p. 178° was 178°. The 2,4-dinitrophenyl sulphide on recrystallisation from alcohol had m.p. 128°. A mixed melting point with authentic 2,4-dinitrophenyl methyl sulphide of m.p. 128° was 128°.

A further portion of the mercuric chloride precipitate after alkali decomposition failed to give any precipitate on aspiration through mercuric chloride (3% w/v). It was also aspirated through chloramine-T solution. This solution, together with that from the original decomposition, on extraction with chloroform and subsequent paper chromatography,[13] both failed to give a spot for sulphidimines. The precipitate in the 2,4-dinitrophenylhydrazine was not examined.

When nitrogen was used as the carrier gas, a precipitate was obtained in the mercuric cyanide tube only. This was treated in the same way as the preceding mercuric cyanide precipitates. It gave on recrystallisation a m.p. of 177°. A mixed melting point with authentic

mercury methanethiol of m.p. 178° was 177–178°. The dinitrophenyl sulphide had m.p. 127°. A mixed melting point with an authentic sample of 2,4-dinitrophenyl methyl sulphide of m.p. 128° was 127–128°.

Discussion

The preliminary investigations showed that fresh potato, undamaged or cut, evolved odours different from those of the cooked material.

The advantage of using capillary columns and the small detector are speed of analysis, good resolution (especially important for low boiling compounds) and the high sensitivity (10^{-12} mole per ml. of carrier gas) of the detector, which enables trace components to be investigated. At present, the main disadvantage of the capillary column technique is that no confirmatory evidence can be obtained from the determination of infra-red or mass spectra, but it is possible to trap the evolving components separately and examine their olfactory characteristics.

A considerable amount of work has been published in recent years on the flavour of heat-treated foods such as coffee[7] and meat,[8] and of fresh foods, e.g., fruit, vegetables[11, 12] and milk.[16] In the cooked foods, there is now evidence to show the importance of sulphur compounds, both mercaptans and sulphides, and carbonyl compounds including both aldehydes and ketones.[7, 8, 17, 18] It is of some consequence to ascertain how these compounds are formed and what controls the relative amounts present, since it is only from this that the reasons for the various flavour differences may be understood.

It has now been shown[19] that the Strecker degradation of methionine by ninhydrin formed the aldehyde methional, methyl mercaptan, dimethyl sulphide, isobutyraldehyde and acrolein. The fact that the degradation goes further than methional is of considerable interest; for the present the mechanism remains obscure. It has been suggested[19–21] that a similar type of breakdown takes place in foods. The diversity of type and structure of the amino-acids found in foods, either free or combined in protein, could account for many of the mercaptans, sulphides, aldehydes or ketones known to be formed on cooking. In this present work the aldehydes isolated (Table I, Fig. 1) could arise from the amino-acids present in potato;[22, 23] thus acetaldehyde, isobutyraldehyde and isovaleraldehyde would arise from the degradation of alanine, valine, leucine, respectively. n-Butyraldehyde would require the presence of norvaline $CH_3{\cdot}CH_2{\cdot}CH_2{\cdot}CH(NH_2){\cdot}COOH$ but this amino-acid has not yet been found in potato. Carbon dioxide and ammonia could also be explained in this way. Altogether some twenty amino-acids have been isolated from potatoes, but in this present work only three of all the possible aldehydes have so far been found. The presence of acetone and methyl isopropyl ketone could not be readily accounted for by the decarboxylation and deamination of any known amino-acid. Acetone could have arisen from a more complex breakdown involving the further oxidation of isobutyraldehyde. The finding of hydrogen sulphide, methyl mercaptan, ethyl mercaptan, dimethyl sulphide and acrolein, suggests a breakdown of amino-acids by some mechanism more complex than that envisaged in the Strecker degradation.

Other workers[24–26] have shown that cysteine may react relatively easily with aldehydes to form either thiazolidine compounds or mercaptals, and with ketones to give mercaptoles, according to the relative amounts of the reactant present. Mizutani also showed that these compounds if heated in aqueous solution yield hydrogen sulphide in differing amounts, according to the nature of the aldehyde or ketone. The thiazolidine compound formed by reacting cysteine with furfural was the most unstable.

Schönberg and co-workers[27, 28] have shown that a large number of compounds, apart from ninhydrin and including several widely distributed in living tissues, will decompose amino-acids when heated. Of the naturally occurring compounds with three carbon to oxygen double bond linkages, dehydroascorbic acid was selected for a preliminary examination in a model system.

In the decomposition of cysteine and cystine by dehydroascorbic acid the absence of precipitates with mercuric chloride and with mercuric cyanide on decomposition of the original black mercuric cyanide precipitate with acid, may be taken as indicating the absence of thiols or sulphides other than hydrogen sulphide. The decomposition of methionine gave methanethiol and

dimethyl disulphide when the decomposition mixture was aspirated with air. When nitrogen was used methanethiol only was obtained. In both instances precipitates were obtained with 2,4-dinitrophenylhydrazine. Methanethiol in the presence of oxygen is known to form dimethyl disulphide. Whether the formation of dimethyl disulphide in this instance is a result of either a direct reaction of methanethiol with oxygen in the liquid phase or vapour, or a more complex series of changes involving the dehydroascorbic acid or a breakdown product from it, is not known.

The failure to detect dimethyl sulphide is of interest since this was formed from methionine when ninhydrin was used for degradation.[19]

The possibility of a pyridoxal[29, 30] cleavage also exists, but so far volatile components do not appear to have been isolated in this reaction.

Since dehydroascorbic acid is known to occur in potatoes[31] its reaction with cysteine and cystine and methionine could account for the formation of the hydrogen sulphide and methanethiol. The hydrogen sulphide could also arise from breakdown of a thiazolidine compound. The origin of the other volatile sulphur compound remains to be elucidated.

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RAPID METHOD FOR THE ESTIMATION OF TOTAL PHOSPHORUS IN SOILS

By R. S. BECKWITH and I. P. LITTLE

Some Queensland soils fail to release the whole of their phosphorus during extraction for 4 h. with boiling hydrochloric acid unless previously ignited. Loss of phosphate from extracts of non-ignited soils is apparently due to titanium oxides which may either adsorb dissolved phosphate during the period of the extraction, or dissolve partially and reprecipitate when the soil extract is diluted and cooled. Pre-ignition renders soil-titanium less soluble and allows complete recovery of phosphorus in soils with boiling acid.

Phosphorus in such solutions can be conveniently determined by the molybdovanadophosphoric acid method after fuming off with perchloric acid. An interfering element (probably cerium) has been encountered in many soil extracts but a reduction treatment readily overcomes this interference.

Introduction

The use of boiling hydrochloric acid as an extractant of soil phosphorus has lost favour with most soil chemists because it often fails to indicate, even roughly, the total phosphorus content of soils (e.g., Tucker[1]). Although Wild[2] used an average figure of 68% for the recovery of total P by boiling hydrochloric acid, the actual recoveries for different soils vary over a wide range. The present work has shown that if soils are ignited (1 h. at 500–550°) before extraction, boiling hydrochloric acid can dissolve the total phosphorus from them. With use also of the molybdovanadophosphoric acid method,[3] in perchloric acid medium[4] (hereafter referred to as the molybdovanadate method), to determine the dissolved phosphate, it has been possible to develop a rapid, convenient method for estimating total phosphorus in soils.

Experimental

Use of the molybdovanadate method on acid extracts of soils.

The application of the molybdovanadate colorimetric method to hydrochloric acid extracts of soils was tested by analysing fifteen extracts previously analysed by a ' molybdenum blue ' method.[5] Comparison of the results is shown in Fig. 1 and agreement is satisfactory except for the two extracts A and B. Reasons for the discrepancy with these extracts are considered later.

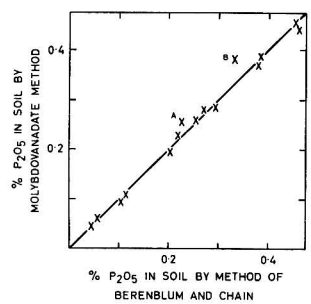

FIG. 1.—*Comparison of determinations of phosphate in hydrochloric acid extracts of soils by the molybdovanadate and by the Berenblum & Chain method*

Reproducibility of acid extracts of soils

Hydrochloric acid extracts (4 h. at boiling with 100 ml. of HCl to 20 g. of soil) of 12 soils were now prepared. These included representatives of the more important soil groups of south-east Queensland and also a number of the soils whose extracts were analysed above. Phosphorus in all 12 soils had been previously determined by the ' molybdenum blue ' method.[5] Only six of the fresh extracts gave phosphate values, by the molybdovanadate method, which agreed satisfactorily with the previous values. With two soils (a krasnozem and a prairie-like

soil) the molybdovanadate method gave results 40–60% larger than those found before. As one of these soils had been analysed previously by both colorimetric methods, on the same soil extract, and the agreement of results was then good, it seemed that the acid extraction procedure failed not only to recover total phosphorus from many soils, but also to recover a reproducible percentage of phosphorus from some soils.

Effect of pre-ignition on the extraction of soil phosphorus

It was observed during routine analysis of soils that dry heating before boiling with hydrochloric acid increased the amount of phosphorus dissolved. Examination of several of the soils giving non-reproducible acid extracts showed that pre-ignition according to Saunders & Williams[6] in some way increased the amount of phosphorus extracted by boiling acid (Table I).

Table I

Extraction of phosphorus from soil samples with and without previous ignition for 1 h. at 500–550°

Soil no.	Great soil group		% P_2O_5 extracted by boiling HCl	
			Without pretreatment	After ignition at 500–550°
B673	Black earth	0–5 in.	0·259	0·345
B229.1	Krasnozem	0–2½ in.	0·352	0·458
B262.1	Prairie-like soil	0–4 in.	0·226	0·467
B261.3	Krasnozem (neutral)	8–14 in.	0·210	0·259
B263.3	Prairie-like soil	11–16 in.	0·493	0·626

Table II

Extraction of phosphorus from a red earth, from Capertee, N.S.W., with boiling hydrochloric acid

Time of extraction, h.	P_2O_5 in extract, g./l.
½	1·61
1	2·08
2	1·29
4	1·05
8	0·84

Loss of phosphate from acid extracts of soils

Many filtered hydrochloric acid extracts of non-ignited Queensland soils have been observed to deposit pale-coloured precipitates when kept, sometimes even overnight. Replicate analyses were made by the molybdovanadate method for phosphorus in such extracts and the results varied depending on the amount of precipitate included in the aliquot. The amounts of phosphate extracted from unignited samples of a red earth were found first to increase and then to decrease with increasing time of extraction (Table II). Furthermore, of 50 mg. of P_2O_5 added to a prairie-like soil before extraction with boiling hydrochloric acid only 12 mg. of P_2O_5 was found in the freshly-filtered extract. Clearly, phosphate can be lost from acid extracts of soils both during the extraction procedure and after filtration and dilution of the extracts.

Qualitative tests on the precipitates from extracts of two soils indicated that titanium was responsible for the loss of phosphate from solution. Subsequently K. Norrish (personal communication) identified one of these precipitates as anatase, and pointed out that its amount seemed too small to attribute the loss of phosphorus to adsorption alone. Ward,[7] however, has also recorded loss of phosphate from soil extracts due to precipitation with titanium and further study of the chemical reaction of titanium and phosphate in pure chemical systems would be of interest.

Effect of pre-ignition on the extraction of titanium

The soils examined in regard to coprecipitation of titanium and phosphate both released more phosphate to boiling hydrochloric acid after ignition. It seemed that pre-ignition might reduce the solubility of soil-titanium compounds. Analyses of extracts of four soils, with and without pre-ignition, showed that the amount of titanium extracted in the latter case was only 10–20% of that extracted in the former (Table III).

Comparison of phosphorus soluble in acid from ignited soils with total phosphorus

To compare the phosphorus soluble in boiling hydrochloric acid from ignited soils with their total P contents, 27 soils were analysed by sodium carbonate fusion.[8] Fig. 2 indicates that the modified acid extraction procedure provides a valid, rapid estimation of total phosphorus in these widely varying soils.

Table III

Solution of titanium from soils before and after ignition

Soil sample (Great Soil Group)	Titanium dissolved (mg./g. soil) from	
	Untreated sample	Ignited sample
Krasnozem	7·7	1·5
Black Earth 0–3 in.	5·3	0·59
Black Earth 48–72 in.	4·9	0·53
Krasnozem (neutral)	6·7	0·48

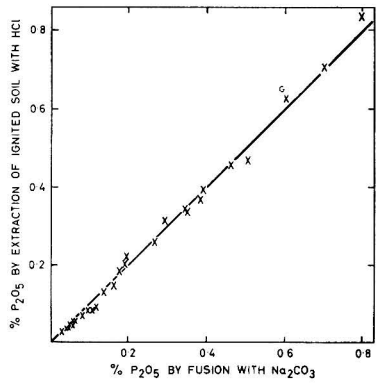

FIG. 2.—*Comparison of estimations of total phosphorus in soils by extraction with hydrochloric acid after ignition and by fusion with sodium carbonate according to Muir*

Finally it may be noted that one soil included in this study came from a soil horizon in which Norrish[9] has reported the occurrence of a highly insoluble phosphate mineral of the gorceixite–florencite sequence. These minerals can apparently be dissolved by the recommended procedure.

Recommended procedure for the rapid estimation of total phosphorus in soils

Reagents

(1) 0·020M-*Ammonium vanadate.*—Dissolve 1·17 g. of ammonium metavanadate in approximately 400 ml. of water, acidify with 25 ml. of 8M-perchloric acid (69 ml. of 70–72% $HClO_4$ diluted to 100 ml.) and dilute to 500 ml.

(2) 0·20M-*Ammonium molybdate.*—Dissolve 35·3 g. of ammonium molybdate tetrahydrate in water and dilute to 1 litre.

(3) *pH Indicator.*—Dissolve 0·1 g. of 2,6-dinitrophenol in 100 ml. of water (pH range 2·2c.–4·4y.).

(4) *Standard phosphate solution.*—Dissolve 1·917 g. of A.R. monopotassium phosphate in water and dilute to 1 litre. This solution contains 1 mg. of P_2O_5 per ml. Also prepare, by dilution, a standard containing 0·1 mg. of P_2O_5 per ml.

Procedure

Weigh 20-g. portions of the soils to be analysed into silica capsules, then stir into the surface of each sample approximately 2 g. of powdered magnesium acetate and heat the capsules in an electric furnace for 1 h. at 500–550°. Cool and transfer the soils to 250-ml. tall beakers and heat in a boiling waterbath for 4 h. with 100 ml. of hydrochloric acid (2 parts of conc. acid to 1 part of water). Stir the suspensions periodically and cover the beakers with watchglasses for much of the extraction period to avoid excessive loss of acid. (Approx. 50 ml. of acid should remain after the 4-h. extraction.) Wash the watchglass and dilute the extract before filtering through a Whatman No. 50 filter paper with gentle suction on a Buchner funnel. Wash the residue on the filter with hot N-HCl and make up the combined filtrate and washings to 250 ml.

Transfer suitable aliquots of the hydrochloric acid extracts (usually 5–10 ml., but containing 0·05–1·0 mg. of P_2O_5) to 100-ml. Kjeldahl flasks and evaporate almost to dryness. Add 2–3 ml. of 70% perchloric acid (2 ml. is sufficient unless the aliquot contains large amounts of solids) and heat until all HCl is expelled and dense white perchloric acid fumes emerge from the neck of the flask. (Some care must be exercised at this stage to avoid over-heating the contents of the flask and forming oxides of iron which can be difficult to redissolve.) Add about 5 ml. water to the cooled digests, followed by a few magnesium turnings, and boil to expel hydrogen from the solutions. Transfer the cooled (colourless) solutions to 50-ml. volumetric flasks keeping volumes of rinse water small so that the total volume of the transferred solution does not

exceed about 25 ml. Neutralise the solutions with aqueous ammonia of about 2N strength to the formation of ferric hydroxide or to end-point (yellow colour) with 2,6-dinitrophenol and acidify soon afterwards with 3·5 ml. of 70–72% perchloric acid. (A constant amount of acid in the range 3·3–3·8 ml. of 70–72% acid, added to all samples and standards, gives satisfactory results.) Add successively 5 ml. of 0·02M-ammonium vanadate and 10 ml. of 0·2M-ammonium molybdate, mixing the solutions after each addition. Make the coloured solutions to volume, mix well, and set aside for at least 30 min. to ensure full colour development. Measure optical densities in a 20-mm. cuvette with a dark blue filter (e.g., Ilford 601), or with spectrophotometers at a wavelength of 4000 Å. Amounts of P_2O_5 present can then be read from a standard curve prepared using 1–10-ml. portions of the dilute standard phosphate solution with perchloric acid, ammonium vanadate and ammonium molybdate added as already described.

Discussion

As pointed out by Piper,[10] the classical hydrochloric acid digestion of soils provides only an empirical measurement of soil phosphorus. However, the present work indicates the cause of the incompleteness of extraction of phosphorus, viz., that hydrolysis of titanium salts causes phosphate to be lost from solution by adsorption or co-precipitation. Although pre-ignition of soils apparently renders soil-titanium compounds less soluble to boiling hydrochloric acid, appreciable amounts may still be dissolved and some precipitation of titanium oxides may occur when the extracts are diluted and stored. It is important, therefore, that aliquots of the hydrochloric acid extracts be taken for analysis without undue delay after filtering. It is an advantage of the present procedure that any precipitated phosphate included in an aliquot of shaken hydrochloric acid extract is redissolved during digestion with perchloric acid.

Two aspects of the analytical procedure may require explanation. The extracts are heated in a boiling waterbath rather than over an electric element or gas burner because ignited soils possess a strong tendency to ' bump ' when boiled. The reason for the treatment with magnesium turnings after the fuming with perchloric acid is to reduce an interfering yellow colour which develops with many soils. As even very pale yellow colours cause interference at the wavelength used for colorimetry, the reducing treatment has now been adopted as a routine precaution with all soil extracts. The interfering element(s) can be reduced with iron, zinc or magnesium metal and traces of ceric ions are regarded as the probable cause of the interference. The poor agreement of the analyses of the soil extracts A and B (Fig. 1) by the molybdovanadate and Berenblum & Chain[5] procedures may have been due to interference of this kind. The results of Table IV show that in high phosphate soils, the amount of interfering substances may be equivalent to as much as 0·1% of P_2O_5 in the soil. Alternatively, some precipitated

Table IV

Determination of phosphorus in three soils by fusion and by the present method with and without destruction of interfering colour by reduction

Soil no.	Muir's method	% P_2O_5 in the sample found by extraction with hydrochloric acid after ignition	
		Without reducing treatment	With reducing treatment
M777	0·704	0·815	0·707
B262.1	0·505	0·576	0·465
B263.3	0·603	0·675	0·626

phosphate may have been determined by the former procedure but not by the latter. Repeat analyses of acid extracts of ignited samples of these soils agreed satisfactorily with analyses by sodium carbonate fusion (Fig. 2).

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ELECTROPHORETIC ANALYSIS OF FLOUR PROTEINS. II.*—Gluten†

By V. L. KOENIG

An investigation of the electrophoretic composition of the gluten proteins in acidic and basic buffers was undertaken. Three main components predominated, although at different distributions, when gluten was analysed in acidic and basic buffers. In addition, there were small amounts of other components—probably some 'salt-soluble' proteins trapped in the gluten. Acidic and basic extracts of the residues from the extraction of flour with 0·1M-sodium chloride yielded components similar to those appearing in gluten. Basic and acidic extracts of flour yielded components similar to those appearing in gluten, and in addition, the 'salt-soluble' proteins and polysaccharides. Adjustment to acid pH of alkaline extracts of flour and the above-mentioned extraction residues did not give electrophoretic patterns identical with those of acidic extracts.

Electrophoretic enantiography was best in the acid buffers. Sodium chloride–hydrochloric acid buffers approached phosphate and aluminium lactate buffers in excellence. Dispersions of gluten in cupric ammonium sulphite were also analysed electrophoretically.

Electrophoretic analyses of gluten from flour defatted with butanol and butanol–ether differed little from those of gluten from intact flour. There was, however, a difference in the electrophoretic analysis of gluten obtained from flour exhaustively extracted with a series of lipid solvents.

Introduction

The proteins of wheat flour have been classified for many years as those soluble and insoluble in neutral salt solutions.[1] The soluble proteins have arbitrarily been said to include the albumins and globulins and the insoluble proteins are essentially what is known as 'gluten' which is very slightly soluble at neutral pH and at ionic strengths of 0·1 or above. At very low ionic strengths (below 0·1) and extremes of pH, i.e., 2–3 and 9–11, appreciable amounts of gluten can be dispersed. Precipitation of gluten by adjusting the pH of acid or alkaline solutions to neutrality apparently results in a gluten possessing more or less its original properties.[2] That the gluten proteins of wheat present a challenging topic for investigation is amply evident by the large volume of literature that has accumulated.

Osborne[1] considered gluten to consist of at least two main fractions, glutenin (insoluble in 70% alcohol) and gliadin (soluble in 70% alcohol). Electrophoretic analyses of gliadin have been reported by Schwert *et al.*[3] and Kondo & Owada,[4] but completely satisfactory patterns

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were not obtained in either investigation. Holme & Briggs[5] later improved the electrophoretic patterns by the addition of urea to the gliadin solutions. Mills[6] maintains there are as many as four electrophoretically distinct proteins in gliadin. More recently, Jones *et al.*[7] and Woychik *et al.*[8] employed aluminium lactate buffer in the electrophoretic analysis of gluten, prepared from butanol-extracted flour washed free of starch with 0·1% sodium chloride. Initial solution of the gluten was in dilute acetic acid. Woychik *et al.*[9] separated certain components of gluten on carboxymethyl cellulose columns. Dimler & Senti[10] have recently reviewed investigations on gluten.

Laws & France[11] made a comparative study of some protein fractions of wheat flour using free electrophoresis. They were unable to observe any striking differences in the varieties studied. Recently, Elton & Ewart[12] applied starch-gel electrophoresis to wheat proteins including gluten and obtained promising results. Gortner *et al.*[13] made a thorough study of the solubility behaviour of wheat proteins in various salt solutions at a variety of ionic strengths. The results indicate rather striking differences for the various salts. Other authors[14–16] have prepared comprehensive reviews on the present status of wheat proteins.

It seems safe to assume that one might obtain a variety of electrophoretic components from gluten depending upon the way in which the gluten is prepared. As gluten is commonly prepared by exhaustive washing of a ball of dough with water to remove starch, it must be considered to be a complex containing more or less of the ' insoluble ' proteins of wheat and in addition some of the soluble proteins of wheat that have been trapped in the dough-ball. An appreciable amount of gliadin may be removed from the dough during the washing process, while there is the strong possibility that considerable denaturation of the gluten proteins may occur during the prolonged manipulative period.

Because of the uncertainty as to what proteins gluten may contain, an investigation of all the extractable proteins in relation to each other could be profitably undertaken. It was decided, therefore, to study the electrophoretic composition of the proteins, both in buffers of alkaline and acid pH at a variety of ionic strengths ($\Gamma/2$), present in glutens prepared in the traditional manner, i.e., exhaustive washing of dough-balls with water to remove starch. Electrophoretic examination was also made of extracts of residues from flour remaining after the removal of soluble proteins, and of alkaline and acid extracts of flour. A comparison between extracts of gluten from intact flours and defatted flours was made. The electrophoretic properties of the ' salt-soluble ' proteins are discussed in another paper.[17]

Experimental

Preparation of gluten

Gluten was prepared from the 0·1M-sodium chloride extraction residues of an unbleached, soft wheat patent flour.[17] The residues were kneaded into dough-balls in running tap water and most of the starch was washed out by prolonged working of the dough-balls in the running water. The final gluten was a rubbery elastic mass which was stored in the frozen state or lyophilised and stored dry.

Glutens were prepared from similar residues from flours which had been defatted with butanol and butanol–ether by repeated suspension in water and centrifugation. In this case the gluten did not form a ball, but remained as a layer on top of the starch layer formed at the bottom of the centrifuge bottle. The gluten layer was ' peeled ' from the starch and collected.

A 0·1M-sodium chloride extraction residue from flour that had been extracted with a series of organic solvents was also examined. The flour was extracted at room temperature three times with eight parts of water-saturated n-butanol to one part of flour, then twice with eight parts of a 2 : 1 chloroform–methanol mixture. The flour residue then contained 0·014% residual lipid.

Preparation of suspensions

Suspensions were made by dispersing 1·5-g. samples of fresh gluten (cut into small pieces) in 30 ml. of 0·01M-acetic acid, 0·01M-potassium hydroxide, or any buffer desired. Similar suspensions were made with weighed quantities of dried gluten or gluten from defatted flour.

In some cases, the 0·1M-sodium chloride extraction residues were suspended directly in the dilute acid, base, or the buffer desired. For the extracts of flour, 25 g. of flour were extracted one or more times with 100 ml. of dilute acid or base.

In addition to the suspension of gluten in alkaline or acid solution, suspensions of gluten were made by the technique of McDermott & Pace,[18] which involves the fission of disulphide bonds by cupric and sulphite ions in the presence of aqueous ammonia with or without urea.

Preparation of buffers

Standard veronal buffer was adjusted from the usual 8·6 to 9·0 by adding aqueous ammonia. The ionic strength was near 0·1. Other buffers were prepared by dissolving the calculated quantity of the salt in distilled water and adjusting the pH with the appropriate alkali (e.g., potassium hydroxide for potassium phosphate) or with hydrochloric acid as necessary. These buffers had ionic strength between 0·01 and 0·1 (see Tables).

Various acid buffers were also preferred (see Table II). Aluminium lactate buffer, pH 3·0, was prepared according to Jones *et al.*[7]

Electrophoretic procedures

The Spinco Model H electrophoresis and diffusion apparatus was used for the electrophoretic analyses. The procedure and interpretation of the results were similar to those previously described.[19] The protein solution was dialysed against an excess of buffer for at least 24 h. and the dialysed samples were clarified by centrifugation with subsequent vacuum filtration through a Celite pad. The electrophoretic mobility (μ) is expressed as 10^{-5} cm.^{2}/sec./V. The electrophoretic results are tabulated to give the percentage distribution (%) for a range of mobilities. If two components occur in the same mobility range, they are listed as two components. Since there are probably considerably more components than can be detected easily in an ordinary electrophoretic pattern, considerable arbitrariness is exercised in interpreting the patterns, but great care has been taken to be consistent. The components of each mobility range are lettered alphabetically for convenience in identification, and those of mobility range A–D are tabulated in one column to conserve space in the Tables. No attempt has been made to correlate components appearing in one buffer with similarly lettered components of another buffer. Especially it is difficult to match components in an alkaline buffer with those in an acid buffer. The proteins migrated towards the anode in alkaline buffers and towards the cathode in acid buffers. Arrows in the figures indicate the direction of migration.

Determination of protein concentration

The protein concentrations of the solutions were determined with a Bausch & Lomb refractometer which had been previously calibrated with solutions of bovine plasma albumin. When there is considerable non-dialysable material other than protein present, the concentration of non-dialysable total solids is obtained rather than protein concentration. This value is obtained for flour extracts and possibly the residue extracts.

Results

Electrophoretic analyses in alkaline buffers

(1) *Ammonium borate buffer*

Typical electrophoretic patterns at ionic strength 0·025 are given in Fig. 1*a* for samples of gluten suspended in dilute aqueous ammonia. The enantiography of the patterns is good. The results in Table I indicate a distribution of at least three main components at $\Gamma/2$ 0·025. As the ionic strength increased, the mobilities of these components decreased. A variety of minor components appeared depending upon the preparation and the ionic strength of the buffer. The second sample listed for this buffer in Table I is for a gluten from flour that had been exhaustively extracted with a series of lipid solvents. The formation of a gluten ball from this material was impossible. A dilute aqueous ammonia extract was therefore made after the 'salt-soluble' proteins had been removed. The solubility of the material was very low and

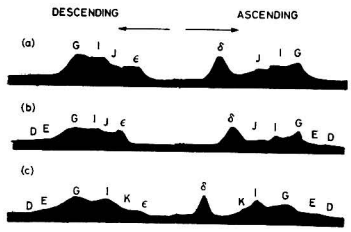

FIG. 1.—*Electrophoretic patterns of gluten in alkaline buffers*
(*a*) Ammonium borate buffer, pH 10.2, $\Gamma/2$ 0·025, 132·5 min., 1·1%, 45° angle
(*b*) Ammonium phosphate buffer, pH 10·3, $\Gamma/2$ 0·05, 86·5 min., 0·5%, 35° angle
(*c*) Potassium phosphate buffer, pH 9·1, $\Gamma/2$ 0·025, 106·7 min., 0·8%, 35° angle

only the minimal concentration for satisfactory electrophoretic analyses was obtained. The distribution of the components is considerably different from that obtained for gluten from intact flour. The enantiography of the analyses was good (no patterns are presented).

(2) *Ammonium phosphate buffer*

Similar analyses were made with ammonium phosphate buffer, pH 10, at ionic strengths of 0·025, 0·05 and 0·10. The symmetry of the patterns at ionic strength 0·025 was so poor that interpretation was impossible. At ionic strengths of 0·05 and 0·10, the enantiography was considerably better. The patterns for the analysis at an ionic strength of 0·05 are shown in Fig. 1*b*. At ionic strength 0·1, less protein (0·3%) dissolved than at ionic strength 0·05 (0·5%). Values in Table I indicate that the distribution of the main components falls into mobility ranges similar to those with ammonium borate buffer. There is a slight shift of mobilities to lower values at the higher ionic strength.

Table I

Electrophoretic results for gluten, residues and flour extracts in alkaline solutions

Material	Concn., g./100 ml.	pH	$\Gamma/2$	Conductivity, ohm^{-1} cm.$^{-1}$	V/cm.	% distribution of components according to mobility range							
						A–D	E	F	G	H	I	J	K
Ammonium borate													
Gluten	1·10	10·2	0·025	0·001330	8·00				49		28	23	
Defatted flour residue	0·18	9·95	0·010	0·000578	13·70		3		25	11	37	18	16
Ammonium phosphate													
Gluten	0·50	10·3	0·05	0·001050	10·10	4	4		50		27	15	
Potassium phosphate													
Dried gluten	1·10	10·0	0·025	0·00105	3·78	4	11		55		20	10	
Gluten from benzene-extracted flour	1·10	10·0	0·025	0·00103	7·71–10·30	3	5		54		26	12	
Gluten from butanol–ether-extracted flour	0·50	10·2	0·025	0·00102	10·40			10	36		24	11	19
Residue (0·01M-KOH)	0·40	10·0	0·025	0·00100	10·60		2	14	14	10	28	19	13
Residue (dil. NH_3)	0·70	10·1	0·025	0·00104	10·20	3	5		36	16	26	7	14
Flour 1st and 2nd extract	0·70	10·4	0·025	0·00107	10·10	4, 7, 7	5	11		8		15	43
Flour 3rd extract	0·80	10·4	0·025	0·00107	9·96				42	34	17		7
Flour 4th extract	0·30	10·1	0·025	0·000973	10·20	2		33		40		9	16

Mobility range: A–D 7·0–11·0; E 6·0–7·0; F 5·0–6·0; G 4·0–5·0; H 3·0–4·0; I 2·0–3·0; J 1·0–2·0; K 0·0–1·0

(3) *Potassium phosphate buffer*

Fig. 1*c* shows a typical electrophoretic analysis of a suspension of gluten in 0·01M-potassium hydroxide using potassium phosphate buffer, pH 10, $\Gamma/2$ 0·025. Enantiography is sufficient

for a satisfactory interpretation, but is not exceptional. The consistency in the distribution of the main components shown in Table I for the gluten samples is fairly good, i.e., the predominating components fall within the same mobility range as for the other alkaline buffers. The distributions for analyses of glutens from benzene-extracted and butanol–ether-extracted flours are consistent with those of the glutens from intact flours, except that the latter gluten contained an appreciable amount of material with zero mobility—presumably polysaccharides.

Residues remaining after the extraction of the ' salt-soluble ' proteins were suspended in 0·01M-potassium hydroxide and in dilute aqueous ammonia. The distributions of the components shown in Table I are somewhat different for the two analyses. Substantial amounts of components H, I and K are present in both series. Dilute aqueous ammonia appears to extract more of the G component, while more of the F and J components are extracted with 0·01M-potassium hydroxide.

The combined first and second extracts of flour with 0·01M-potassium hydroxide have a substantial amount of the polysaccharide fraction (component K), but appreciable amounts of the components occurring in gluten (G, H and I) do not appear until the third and fourth extracts. Component F appears to the greatest extent in the fourth extract. Patterns of the analysis for the combined first and second extracts of flour are shown in Fig. 3*a*. These will be discussed later in connexion with analyses made at pH 3·0 in phosphate buffer.

Electrophoretic analyses in acid buffers

(1) *Aluminium buffers*

Of the aluminium buffers used, the lactate seemed to be the most satisfactory from the standpoint of resolution and enantiography (see Fig. 2*a*). The percentage distributions of the components are shown in Table II. Analyses were also made with aluminium chloride–hydrochloric acid and aluminium chloride–lactic acid buffers at pH near 3·0. The distributions of the main components, although slightly different, occur in the same mobility ranges for the two buffers. The enantiography was satisfactory although not so good as with aluminium lactate. With aluminium chloride–hydrochloric acid, less protein (0·3%) dissolved at $\Gamma/2$ 0·1 than at $\Gamma/2$ 0·05 (0·5%). At $\Gamma/2$ 0·1 the mobilities were shifted to lower values than at $\Gamma/2$ 0·05.

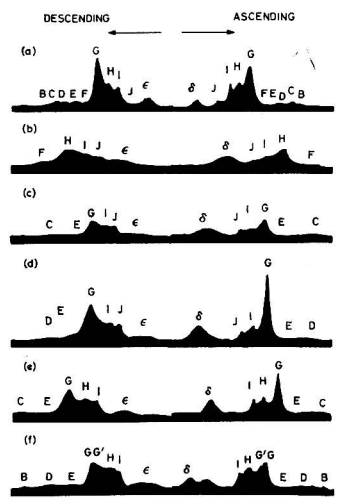

FIG. 2.—*Electrophoretic patterns of gluten in acid buffers*
(*a*) Aluminium lactate, pH 3·1, 114·9 min., 0·9%, 45° angle
(*b*) Acetate buffer, pH 4·0, $\Gamma/2$ 0·05, 243·6 min., 0·28%, 35° angle
(*c*) NaCl–HCl, pH 2·1, $\Gamma/2$ 0·025, 213·4 min., 0·4%, 35° angle
(*d*) NaCl–HCl, pH 3·0, $\Gamma/2$ 0·025, 166·4 min., 0·6%, 35° angle
(*e*) Phosphate, pH 3·3, $\Gamma/2$ 0·025, 111·1 min., 0·4%, 35° angle
(*f*) $ZnCl_2$-lactic acid, pH 2·9, $\Gamma/2$ 0·05, 118·3 min., 0·3%, 35° angle

Table II

Electrophoretic results for gluten in acid solutions

Material	Concn., g./100 ml.	pH	$\Gamma/2$	Conductivity, ohm^{-1} cm.$^{-1}$	V/cm.	% distribution of components according to mobility range							
						A–D	E	F	G	H	I	J	K
Aluminium lactate													
Gluten	0·90	3·10	0·10	0·000980	8·10	2, 4, 2	5	3	47	18	13	6	
$AlCl_3$–HCl													
Dried gluten	0·50	3·10	0·05	0·001660	7·97			48	18	22	12		
$AlCl_3$–lactic acid													
Gluten	0·40	3·20	0·05	0·001660	7·97	3, 1	2	32	21	22	19		
Acetate buffer													
Gluten	0·28	4·00	0·05	0·002060	5·12			15		47	24	14	
NaCl–HCl													
Gluten	0·40	2·10	0·025	0·00375	3·52	4	6		49		27	14	
Gluten	0·60	3·00	0·025	0·00184	5·76	6	6		61		18	9	
Gluten	0·60	3·10	0·025	0·00184	5·76	8			63		19	10	
Zinc chloride–lactic acid													
Gluten	0·30	2·90	0·05	0·00219	7·86	3, 4	5		32, 18	23	15		
Zinc acetate–acetic acid													
Dried gluten	0·60	3·50	0·10	0·00186	7·12		2	2	1	40	21	12	18, 2
Zinc chloride–hydrochloric acid													
Dried gluten	0·3–0·4	3·30	0·05	0·00218	7·89	6	6		51	23	14		

(Mobility ranges A–K as in Table I)

(2) *Acetate buffer*

Patterns of satisfactory symmetry using acetate buffer, pH 4·0, $\Gamma/2$ 0·05 are shown in Fig. *2b*. Although the enantiography was less than satisfactory at $\Gamma/2$ 0·01, the patterns could still be interpreted. The distribution of the components is shown in Table II. The protein concentration was insufficient at $\Gamma/2$ 0·1 for a satisfactory analysis.

(3) *Sodium chloride–hydrochloric acid buffers*

A number of electrophoretic analyses on gluten were conducted using sodium chloride–hydrochloric acid buffers at two pH and three values of $\Gamma/2$. At pH 2·1, there was satisfactory enantiography at all the ionic strengths studied. A typical set of patterns is shown in Fig. *2c*, and the distribution of components in Table II. With an increase in ionic strength, the enantiography was maintained, although there was a shift in the distribution of the components and in the mobilities to lower values. The solubility of the protein tended to decrease as the ionic strength increased. At pH 3·0, the enantiography was equally good, and the distribution of the components was similar to that of the analyses made at pH 2·1. Typical patterns are given for an analysis at pH 3·0, $\Gamma/2$ 0·025 in Fig. *2d*. Distributions in Table II for two analyses at $\Gamma/2$ 0·025 show very good agreement. Shifts in mobility and distribution with ionic strength occurred similar to those found at pH 2·1.

(4) *Zinc buffers*

The patterns for the analysis with zinc chloride–lactic acid buffer are shown in Fig. *2f*. The enantiography is satisfactory and similar to the other analyses with zinc ion. The concentration anomalies are not symmetrical, two appear in the ascending pattern and one appears on the descending. The distribution results are given in Table II. Zinc acetate–acetic acid buffer, particularly at low ionic strength ($\Gamma/2$ 0·025) around pH 3·0, had very good solvent properties for gluten. At ionic strength of 0·1, zinc acetate–acetic acid buffer produced enantiographic patterns at a protein concentration of 0·6%. The concentration anomalies were asymmetrical and similar to those shown for zinc chloride–lactic acid buffer. There may

have been a formation of zinc–protein complexes. Increase in ionic strength offered no improvement. With zinc chloride–hydrochloric acid buffer, the enantiography was satisfactory at an ionic strength of 0·05. When the ionic strength was increased to 0·1, the solubility of the protein decreased and the distribution of the components was altered.

(5) *Phosphate buffer*

An extensive series of analyses was made in phosphate buffer at pH 3·0 at three ionic strengths, for comparison with the alkaline phosphate buffer, pH 10·0, $\Gamma/2$ 0·025. At optimal protein concentration (0·4–0·6%) patterns showed good enantiography (see Fig. 2*e*). The symmetry of these patterns is similar to that obtained at other ionic strengths. The quantitative values are given in Table III. The first set of analyses were made on gluten samples that had been suspended in 0·01M-hydrochloric acid and subsequently dialysed against the buffer. The other determinations with this buffer were on suspensions in 0·01M-acetic acid with subsequent dialysis against the buffer. The mobilities of the predominating components for gluten suspended in 0·01M-hydrochloric acid were shifted to lower values than for suspensions in 0·01M-acetic acid. Electrophoretic analyses of the freeze-dried gluten and the gluten defatted with benzene present no striking differences. Small differences were observed between analyses of fresh gluten and a freeze-dried gluten at $\Gamma/2$ 0·025. The analysis for gluten from butanol-extracted flour is very similar to that for fresh gluten from intact flour at $\Gamma/2$ 0·025. With increase in ionic strength, there was a shift towards lower mobilities for the major components. Solubility was quite good even at ionic strength 0·10.

Analyses of 0·01M-acetic acid extracts of the residues remaining after the removal of the salt-soluble ' proteins with 0·1M-sodium chloride gave patterns of excellent enantiography. The distribution and mobilities of the major components are slightly different from those found

Table III

Electrophoretic results for gluten, residues and flour extracts in acid solution
(phosphate buffer)

Material	Concn., g./100 ml.	pH	$\Gamma/2$	Conductivity, ohm^{-1} cm.$^{-1}$	V/cm.	% distribution of components according to mobility range							
						A–D	E	F	G	H	I	J	K
(1) Initially suspended in 0·01M-HCl													
Dried gluten	1·20	3·00	0·025	0·00152	2·63	8	6			67	12	7	
Dried gluten from benzene-extracted flour	1·30	3·00	0·025	0·00154	8·60	10	11			62	10	7	
(2) Initially suspended in 0·01M-acetic acid													
Gluten	0·80	3·00	0·025	0·00154	8·65	4	3		62		18, 13		
Dried gluten	0·40	3·30	0·025	0·00143	9·26	1	2		57	24	16		
Gluten from butanol-extracted flour	0·80	3·20	0·025	0·00151	8·80	3	5		62	16	14		
Dried gluten	0·90	3·20	0·050	0·00264	6·77		2	2		65	18	11	2
Residue	0·30	3·20	0·050	0·00259	6·64	3			33	28	21		4, 11
Residue, alkaline to acid	0·50	3·20	0·050	0·00262	6·58	2	4	9		29	20, 14	2	6, 14
Flour	1·20	3·30	0·050	0·00266	6·47	1, 8	4		4	29	15	9	4, 26
Flour, alkaline to acid	0·50	3·20	0·050	0·00274	6·28	1, 7	5	6	12	30	9	3	5, 22
Flour 1st and 2nd extract alkaline to acid	0·60	3·10	0·050	0·00266	6·47	3, 7, 10, 9	10	2			8	5	46
Flour 3rd extract alkaline to acid	0·40	3·10	0·050	0·00266	6·47						45	31	18, 6
Flour 4th extract alkaline to acid	0·50	3·20	0·050	0·00260	6·62			3	5	62	10		5, 15

(Mobility range A–K as in Table I)

in gluten. A component having zero mobility was found—presumably polysaccharide which remained after the saline extraction. The analyses for residues originally suspended in 0·01M-potassium hydroxide with subsequent adjustment of pH to 3·0 by adding acetic acid and dialysis against phosphate buffer, pH 3·0, $\Gamma/2$ 0·05, are listed in Table III. The distribution of components as well as the mobilities are different from those for the residues originally suspended in 0·01M-acetic acid. The polysaccharide component seems slightly larger.

The patterns for a 0·01M-acetic acid extract of flour are shown in Fig. 3*c*. The enantiography is excellent. The results in Table III indicate that there is a large quantity of the components (H, I, J) occurring in gluten. In addition, the polysaccharide component (K) is large, and there is a quantity of faster components normally extracted by 0·1M-sodium chloride. The next analysis is on a dilute aqueous ammonia extract of flour after adjustment of pH to 3·0 and dialysis against phosphate buffer, pH 3·0, $\Gamma/2$ 0·05. The distribution of the components is different from that in the 0·01M-acetic acid extract of flour. The polysaccharide component (zero mobility) is appreciable. The last three analyses are on a combined first and second, the third, and the fourth extracts, respectively, of flour with 0·01M-potassium hydroxide, subsequently acidified and dialysed against phosphate buffer, pH 3·0, $\Gamma/2$ 0·05. Most of the gluten components are not obtained until the third and fourth extracts. The distribution of components certainly is different from that in the 0·01M-acetic acid extracts. The electrophoretic patterns are given in Fig. 3. Satisfactory enantiography was obtained in the patterns of the acidified alkaline extracts.

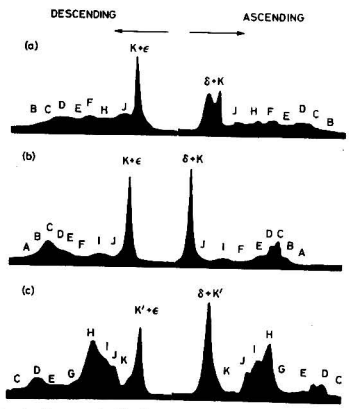

FIG. 3.—*Electrophoretic patterns of alkaline and acid extracts of flour in alkaline and acid potassium phosphate buffers*

(*a*) pH 10·4, $\Gamma/2$ 0·025, 75·1 min., 0·7%, 35° angle
(*b*) pH 3·1, $\Gamma/2$ 0·05, 119·8 min., 0·6%, 35° angle [after extract (*a*) was adjusted to pH 3·0]
(*c*) pH 3·3, $\Gamma/2$ 0·05, 159·4 min., 1·2%, 35° angle

Electrophoretic analyses of gluten

(1) *Dispersed in cupric ammonium sulphite*

Samples of gluten which had been suspended using cupric and sulphite ions in the presence of aqueous ammonia were analysed in veronal and borate buffers. It is necessary to maintain the pH near 10 in order that solution be maximal. When the pH is appreciably below 10, material precipitates. Suspension is faster when 8M-urea is used in conjunction with the cupric and sulphite ions, but with urea an appreciable amount of starch seems to be solubilised. The electrophoretic results for analyses conducted both in veronal buffer and ammonium borate buffer are listed in Table IV. The first analysis is on the solution resulting from the dialysis of the sulphite complex against veronal buffer containing aqueous ammonia at pH 9·26. During dialysis, a gel precipitated which dissolved on the addition of a small amount of ammonia.

Table IV

Electrophoretic results for cupric sulphite dispersions of gluten dialysed against veronal and borate buffers

Material	Concn., g./100 ml.	pH	$\Gamma/2$	Conductivity, ohm^{-1} cm.$^{-1}$	V/cm.	% distribution of components according to mobility range					
						E	F	G	H	I	K
Veronal buffer											
Gluten	0·816	9·26	0·10	0·00386	5·48		7	22	58	13	
Gluten	0·3–0·4	9·50	0·10	0·00400	5·28				8, 80	12	
Precipitate (pH 8·5)	0·80	10·10	0·10	0·00408	5·18–6·48			2	12, 79	7	
Supernatant	0·60	9·15	0·10	0·00403	5·25–6·55	5	27	14		52	2
Ammonium borate											
Gluten	0·80	9·30	0·10	0·00242	8·74		25		54	21	

(Mobility ranges E–K as in Table I)

The second analysis represents the result of adding the dissolved gel to the material of the first analysis. The percentage distributions for the two analyses suggest that the gelatinous precipitate was largely the H component. In order to verify this observation, the pH of the cupric sulphite suspension of gluten represented by the second analysis was adjusted from 10·0 to 8·5. The precipitate formed was subsequently dialysed against veronal buffer, containing ammonia, at pH 10·0. The supernatant was also dialysed against veronal buffer containing ammonia at pH 9·15. The third analysis is for the precipitate and indicates a concentration of the H component ($\mu = 3{\cdot}14$). The fourth analysis, for the supernatant, indicates distribution among five components. The electrophoretic patterns for first and third analyses are shown in Fig. 4. Since the pattern for the third analysis shows two components in the mobility range for the H component, the major component is designated H′ and the minor component, H, for identification purposes. Enantiography is good in both analyses. The last analysis in ammonium borate buffer is on the same preparation used in the first analysis. Since the patterns were similar to those for the first analysis, no pictures are included. There was a slight shift in the distribution (Table IV), but the amount of H component seemed to be nearly the same for both buffers.

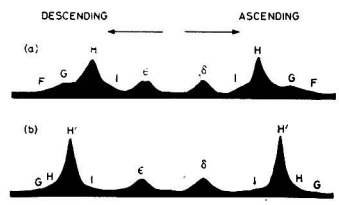

FIG. 4.—*Electrophoretic patterns for cupric sulphite dispersions of gluten dialysed against veronal buffer*
(*a*) pH 9·26, $\Gamma/2$ 0·1, 245·5 min., 0·8%, 35° angle
(*b*) pH 10·1, $\Gamma/2$ 0·1, 291·1 min., 0·8%, 35° angle

Discussion

The primary purpose in this investigation has been to determine the electrophoretic behaviour of the proteins of gluten in various acid and alkaline buffers. The electrophoretic investigations have been performed on materials that were suspended in dilute alkali or dilute acid. No attempt has been made to account for any protein that might not be dispersed under the conditions employed. Incidental to the electrophoretic analyses has emerged information regarding the solubility of the proteins in the various buffers. This has come largely from refractive index measurements and from the electrophoretic patterns themselves. Differences in distribution of the components is due in great measure to differences in the solubility of the proteins in the various buffers and these differences are manifested in the patterns. The mobility values tended to decrease with increase in ionic strength for a given pH in both alkaline and acid buffers.

The electrophoretic analysis of gluten suspensions in 0·01M-potassium hydroxide and dilute aqueous ammonia with alkaline buffers produced patterns which are enantiographic.

Ammonium borate was a highly satisfactory buffer, but it was necessary to maintain the pH near 10 since solubility rapidly decreases when the pH is slightly lowered. Although good symmetry was obtained, it would appear that the resolution is less distinct than at acid pH. Resolution improved with ionic strength, but the boundaries seemed more diffuse than those produced when acid buffers were used. Flour extracts with dilute aqueous ammonia were analysed in ammonium phosphate buffers, but the patterns were too poor for interpretation.

The use of potassium phosphate buffer, pH 10·0, $\Gamma/2$ 0·025, gave symmetrical patterns, but they were more diffuse and less sharply resolved than when acid buffers were used. This buffer afforded a basis for the direct comparison of electrophoresis at acid pH using phosphate buffers in both cases. At alkaline pH, there seemed to be a distribution into three main components with greater proportion in two components. At acid pH, there was usually a preponderance of one component compared with the two lesser components. The first two extracts of flour with 0·01M-potassium hydroxide removed the usual soluble components together with small amounts of those components occurring in gluten. With the third and fourth extracts more of the components appearing in gluten were present in the patterns in varying amounts. Since the pH was not adjusted during the 0·01M-potassium hydroxide extraction, it is to be expected that sufficient alkalinity would not develop to remove most of the components occurring in gluten until the third and fourth extracts.

In general, electrophoretic analyses conducted in acid buffers (pH 2·0 or 3·0) had the more satisfactory patterns. Aluminium lactate gave patterns with excellent resolution and enantiography, and suspended a good concentration of protein. The use of aluminium chloride gave acceptable enantiography. While phosphate buffer and sodium chloride-hydrochloric acid gave good enantiography and resolution, the acid phosphate buffer proved superior, and at least equal to aluminium lactate in excellence. Zinc salts offered some promise, especially from the standpoint of solubilisation. Phosphate buffer, pH 3·0, $\Gamma/2$ 0·05, proved satisfactory for routine examination of the components appearing in gluten. The final conditions for routine study consisted of suspending the preparation in 0·01M-acetic acid and subsequent dialysis against phosphate buffer, pH 3·0, $\Gamma/2$ 0·05. The electrophoretic analyses of gluten in acid solutions were similar in resolution and enantiography to those reported by Jones *et al.*[7]

The extracts of flour with 0·01M-acetic acid contained the components appearing in gluten in good concentration. Sometimes it was necessary to make two extracts on the flour or to adjust the pH to 3·0 to remove all the extractable protein. Alkaline extracts of flour with either 0·01M-potassium hydroxide or dilute aqueous ammonia, with subsequent adjustment of pH from 11·0 to 3·0, did not give patterns in phosphate buffer, pH 3·0, $\Gamma/2$ 0·05, typical of the acid extracts. Whether this difference was due to difference in material extracted or to alteration of protein at alkaline pH has not been determined. Blish[2] and Csonka & Horn[20] report that alkali produces irreversible changes in gluten. When the residues from the 0·1M-sodium chloride extracts of flour were suspended in either alkali or acid, suspension occurred less readily than when flour was extracted directly with acid or dilute alkali.

The cupric ammonium sulphite technique for dispersing gluten produces protein derivatives. While these suspensions can be analysed electrophoretically, there is some uncertainty as to what changes have been produced in the protein. The electrophoretic patterns, while symmetrical, do not compare with those obtained on suspensions of gluten in dilute aqueous ammonia when analysed in the same buffer. Since appreciable amounts of gluten can be suspended in dilute alkaline or acid solutions, it was felt that the electrophoretic analyses using these suspensions might be more indicative of the true nature of gluten.

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ELECTROPHORETIC ANALYSIS OF FLOUR PROTEINS FROM VARIOUS VARIETIES OF WHEAT*

By J. J. KELLEY and V. L. KOENIG

Extracts (0·1M-sodium chloride) of fifteen samples of flour, from six types of wheat, have been analysed by moving boundary electrophoresis. The gluten proteins and 0·01M-acetic acid extracts of the flours were also examined. Electrophoretic enantiography was quite good for all buffers used.

Seven to eleven electrophoretic components were observed for the gluten protein extracts depending on the type of wheat and the extent of resolution. Analyses of the gluten and the acetic acid extracts from flour could be grouped into three categories typified by the Hard Red Spring wheats, the Hard Red Winter wheats and the Durum wheat. Considerably more material was extracted with 0·01M-acetic acid from flour than from gluten, including fast migrating (' salt-soluble ') and polysaccharide material. In general, patterns for the 0·01M-acetic acid extracts of gluten could be superimposed on the patterns for the 0·01M-acetic acid extracts of flour. The good reproducibility of the electrophoretic method was demonstrated by three analyses of glutens from a cake flour.

Differences in component distribution were observed for the 0·1M-sodium chloride extracts of flour when analysed in veronal buffer, at least ten components being observed. Greater amounts of slower migrating components were noted in the Hard Red Winter wheats and in the Club wheat than in the Hard Red Spring wheats. Flour samples, analysed in borate buffer, could be divided into four general groups on the basis of their electrophoretic patterns.

No differences were observed in the electrophoretic analyses of proteins from bleached and unbleached cake flour.

Introduction

The wide divergence among varieties of wheat in their physical and chemical properties has concerned chemists for some time. The unique properties of the gluten proteins, in particular, have made them a choice for most active investigation.[1–3] Jones *et al.*[4] and Cluskey *et al.*[5] have examined by moving boundary electrophoresis the gluten components from a number of wheats but found little difference among the bread wheats studied. Significant differences

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were noted, however, between Durum and bread wheats. Simmonds & Winzor[6] applied column chromatography to the separation of the gluten components, but again, no outstanding differences were observed among the wheat varieties investigated. Starch-gel electrophoresis with aluminium lactate buffer has been used to study cereal proteins by Elton & Ewart,[7] who found significant pattern differences when glutens from various varieties of wheat were analysed.

Material extracted from flour with water or dilute salt solutions has also been investigated for possible differences among the wheat varieties. Laws & France,[8] by moving boundary electrophoresis, compared water extracts of three flours in citric acid–disodium phosphate buffer, pH 2·15, $\Gamma/2$ 0·03, and obtained similar electrophoretic patterns for all three flours. Pence *et al.* have reported differences in the salt-soluble flour proteins based on their amide nitrogen and tryptophan content,[9a] and by their electrophoretic behaviour on filter paper.[9b] Free electrophoresis studies with aluminium lactate buffer, pH 3·2, by Cluskey *et al.*,[5] showed no notable distributional differences in dilute sodium chloride extracts of hard and soft wheat flours. Starch-gel electrophoresis studies by Elton & Ewart[7] also gave similar albumin and globulin patterns for eight wheat varieties.

Work in this laboratory has also been concerned with the electrophoretic behaviour of wheat flour proteins.[10, 11] Gluten proteins and 0·01M-acetic acid extracts of flour yielded patterns which were well resolved and enantiographic when analysed in phosphate buffer, pH 3·2, $\Gamma/2$ 0·05. Veronal buffer, pH 8·6, $\Gamma/2$ 0·1, and borate buffer, pH 9·1, $\Gamma/2$ 0·1, were found to be most useful for the analysis of the salt-soluble proteins. It is the purpose of this paper to discuss the electrophoretic behaviour of proteins extracted from fourteen different wheat flours with these three buffers.

Materials and methods

Flour from six classes of wheat were analysed in this survey; namely, Hard Red Spring, Hard Red Winter, Soft Red Winter, White, Club, and Durum. These flours, experimentally milled, had extraction values between 62 and 70%. Their respective protein contents are shown in Table I. A bleached and an unbleached cake flour (Cake flour 1) and another cake flour (Cake flour 2), used to check the reproducibility of the electrophoretic method, were also analysed as part of this study.

Table I

Flour samples used in survey

(14% moisture basis)

	Protein, %
Hard Red Spring wheats	
Willet	14·0
Selkirk	14·0
Lee	15·2
Thatcher	12·2
Hard Red Winter wheats	
Itana	11·2
Wichita	7·8
Bison	11·0
Columbia Tacoma	8·4
White wheats	
Michigan White*	7·1
Semi-Dwarf	7·3
Brevor	7·5
Club wheat	
Omar	5·2
Durum wheat	
T-mix Durum*	12·3
Soft Red Winter wheats	
Cake flour 1	8·4
Cake flour 2	8·0

* A blend of several varieties

The procedures for the preparation of 0·1M-sodium chloride extracts of flour, 0·01M-acetic acid extracts of gluten and flour, and the buffers for electrophoretic analysis were described previously.[10, 11] A Bausch & Lomb refractometer was used to determine concentrations of the total non-dialysable solids in the extracts.

A Spinco model-H electrophoresis and diffusion apparatus was used for the electrophoretic analyses. The procedure and interpretation of the data were the same as reported previously.[10]

Electrophoretic pictures for the sodium chloride extracts were taken when the fastest moving component had moved across the field. Pictures were taken, in most cases, at two time intervals for the gluten proteins and the acetic acid extracts. The first pictures were taken when the fastest component had moved across the field. The second pictures were taken after the fast components had left the field, thus giving optimal resolution for the slower migrating, gluten components.

The numbers and letters above the peaks have been assigned as an aid in identifying the components described in the tables. Any particular component resolved in one buffer system, however, is not necessarily identical with the same numbered component resolved by another buffer.

Results and discussion

The gluten proteins

The amounts of gluten were noticeably smaller in the flours of low nitrogen content as judged by the size of the gluten ball formed. No striking differences were apparent in the physical appearance of the gluten balls from the various varieties of wheat with the exception that the gluten from the bleached flour was whiter. In Fig. 1 are presented the electrophoretic patterns for the gluten and flour suspensions of Thatcher wheat in 0·01M-acetic acid, taken at two time intervals. The second pictures afford greater resolution of the gluten components; i.e., the slower migrating components. Electrophoretic patterns for the glutens of other classes of wheat are shown in Fig. 2. The components have been lettered using the mobility values as the guide; i.e., components having similar mobilities are given the same letter in this Figure. The primed letters represent mobilities less than those for the unprimed letters, but greater than those for the next letter. The components present in larger amounts seem to resolve consistently and give dependable mobility values. The components present in smaller amounts are more difficult to interpret and hence have less consistent mobility values. It should be emphasised that the interpretation of the electrophoretic patterns is arbitrary. Actually, there is probably a much larger number of components than have been indicated. Every effort has been made to be consistent in the interpretation of all the patterns with the hope that relatively small differences may be detected.

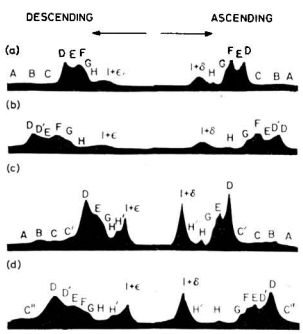

FIG. 1.—*Electrophoretic patterns of* 0·1M-*acetic acid extracts of Thatcher gluten and flour in phosphate buffer* (pH 3·0, $\Gamma/2$ 0·05)

Concentrations: gluten, 0·5%; flour, 1·2%. Diaphragm angle, 35°

(*a*) Thatcher gluten: 150·9 min.
(*b*) ,, ,, : 257·5 min.
(*c*) Thatcher flour: 146·7 min.
(*d*) ,, ,, : 253·5 min.

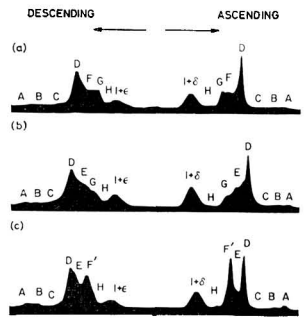

FIG. 2.—*Electrophoretic patterns of* 0·01M-*acetic acid extracts of various glutens in phosphate buffer* (pH 3·0, $\Gamma/2$ 0·05)

Concentrations: Bison, 0·6%; Michigan White, 0·7% T-mix Durum, 0·6%. Diaphragm angle, 35°

(*a*) Bison: 163·8 min.
(*b*) Michigan White: 173·7 min.
(*c*) T-mix Durum: 157·6 min.

In Table II are presented the mobility and percentage distribution results for the gluten components as interpreted in the first pictures. The components are arranged according to mobility into twelve categories, and are lettered corresponding to those in Figs. 1 and 2. The ranges of conductivities and volts per cm. are also included. Table III contains the mobility and percentage distribution results for the glutens as interpreted in the second pictures. Considerably greater resolution is accomplished by allowing the faster boundaries to migrate out of the field and the principal components of gluten to extend to the limits of the field. The distribution results for components observed in the second picture are given in Table III. The amounts of components, A to C, observed in the first picture are included in the last column of the table. The greater resolution obtained in the second pictures resulted in slight shifts in the mobility values.

Table II

Electrophoretic data for glutens from various varieties of wheat

(first picture)

Phosphate buffer, pH 3·0, $I/2$ 0·05, K = 2480–2640 μmhos/cm., 6·53–6·94 V/cm.

Peak	A		B		B′		C		D		D′		E		F		F′		G		H		I	
Variety	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%
Hard Red Spring wheats																								
Willet	7·06	3					4·82	3	3·82	21	3·27	10	2·91	8	2·16	33					1·11	4	0	18
Selkirk	7·84	4					4·53	3	3·46	26			2·85	10	2·19	31			1·53	9	0·91	3	0	14
Lee	7·53	4	6·16	2			4·78	2	3·51	33			2·78	11	2·20	22			1·55	9	0·97	1	0	16
Thatcher	7·76	2	6·58	3			4·80	3	3·52	29			2·77	10	2·22	26			1·60	9	1·09	3	0	15
Hard Red Winter wheats																								
Itana	7·45	3					4·62	2	3·75	50					2·46	16			1·86	13	1·12	1	0	15
Wichita	7·38	1	6·37	1			4·99	1	3·61	53					2·38	15			1·74	14	0·97	1	0	14
Bison	7·33	1	6·46	1			5·09	3	3·50	47					2·36	16			1·67	13	0·87	3	0	16
Columbia Tacoma	7·43	1			5·77	1	4·62	1	3·65	43					2·57	22			1·78	13	1·01	3	0	16
White wheats																								
Michigan	7·08	2	5·99	2			4·76	3	3·55	35			2·75	23					1·76	13	1·01	2	0	20
Semi-Dwarf	7·40	4	6·51	2	5·11	3			3·63	39					2·44	20	1·85	17			0·97	2	0	13
Brevor	7·34	2	6·47	3			4·80	4	3·50	27	2·82	13			2·28	20			1·71	14	0·94	1	0	16
Club wheat																								
Omar	7·48	5	6·36	3			4·88	4	3·66	30	3·02	9			2·39	20	1·83	7	1·46	5	0·91	2	0	15
Durum wheat																								
T-mix Durum	7·15	2	6·28	2			4·59	1	3·47	34			2·68	10			1·99	36			0·84	1	0	14
Soft Red Winter wheat																								
Cake flour 1 unbleached	7·54	3	6·55	3	5·77	2	4·93	3	3·80	41					2·45	22			1·71	11	1·01	1	0	14
Cake flour 1 bleached	7·00	5			5·72	3	4·76	3	3·51	44					2·35	19			1·58	9	0·95	4	0	13
Cake flour 2			6·96	6	5·54	4	4·62	3	3·46	38					2·44	20			1·63	14	0·92	2	0	13
Cake flour 2	7·02	5			5·82	4	4·76	3	3·55	39					2·48	20			1·65	13	0·86	1	0	15
Cake flour 2	7·30	6	6·22	4	5·00	3			3·63	36					2·51	22			1·72	14	0·91	2	0	13

The electrophoretic patterns for Thatcher in Fig. 1 are typical for the Hard Red Spring wheat class. In Table II, the distribution and mobility results present a consistent pattern with the exception of slight differences in the gluten from Willet wheat flour. In Willet, component D′ is resolved, but is not apparent in the other varieties. On the other hand, component G is absent, but is present in the other varieties of this class. Component B is absent in both Willet and Selkirk or is in insufficient concentration for adequate resolution. Furthermore, in the second picture (Table III), component G appears for Willet, thus diminishing the amounts of components F and H remaining. With the additional resolution of the second picture, Willet appears less different from the other varieties. Presumably components A–C are the ' salt-soluble ' proteins which were trapped in the gluten ball.[4, 5, 11] Components D–G represent the main constituents of gluten. Component I represents presumably small amounts of polysaccharides trapped in the gluten and is superimposed on the concentration anomaly. On further resolution, component D of Table II produces in addition to itself component D′ of Table III for Selkirk, Lee and Thatcher flours. The presence of component F′ for Selkirk and the absence of component E for Lee are additional differences apparent in the second picture.

The patterns for Bison in Fig. 2 are typical for the Hard Winter wheats and the results

Table III

Electrophoretic data for glutens from various varieties of wheat

(As Table II, second picture)

Variety	Peak D μ	Peak D %	D′ μ	D′ %	E μ	E %	F μ	F %	F′ μ	F′ %	G μ	G %	H μ	H %	I μ	I %	A-C %
Hard Red Spring wheats																	
Willet	3·77	25	3·25	12	2·81	8	2·22	20			1·72	9	1·00	2	0	16	8
Selkirk	3·77	21	3·29	7	2·96	8	2·60	19	2·20	14	1·72	8	0·90	2	0	14	7
Lee	3·65	31	3·01	12			2·44	22			1·77	10	0·85	1	0	15	9
Thatcher	3·74	21	3·25	10	2·87	10	2·42	22			1·84	13	0·98	3	0	14	7
Hard Red Winter wheats																	
Columbia Tacoma	3·78	40			2·70	22			1·95	15			1·01	3	0	17	3
White wheats																	
Michigan	3·55	37			2·72	13	2·26	9	1·93	5	1·57	7	0·90	1	0	21	7
Semi-Dwarf	3·68	30	2·97	11	2·54	13	2·18	10	1·83	10	1·45	2			0	15	9
Brevor	3·62	26	3·18	9	2·87	5	2·47	19	1·87	14			0·92	1	0	16	10
Club wheat																	
Omar	3·57	31	2·97	9			2·44	18	2·02	9	1·59	7	0·87	1	0	13	12
Durum wheat																	
T-mix Durum	3·63	28	3·19	8	2·83	8	2·14	29			1·54	5	0·77	2	0	15	5
Soft Red Winter wheats																	
Cake flour 1 unbleached	3·67	35	3·07	10			2·46	14	2·00	7	1·65	9	1·07	1	0	15	9
Cake flour 1 bleached	3·62	35	3·03	12			2·56	10	2·12	9	1·60	10	0·92	2	0	14	8
Cake flour 2	3·62	33	3·00	9			2·49	13	2·07	7	1·67	10	1·07	1	0	14	13
Cake flour 2	3·54	32	2·92	12			2·42	11	1·98	8	1·60	9	0·92	1	0	14	13
Cake flour 2	3·75	28	3·10	13			2·62	14	2·16	7	1·76	12			0	13	13

in Table II are consistent for this class. Component D predominates while components F and G appear in lesser amounts. The faster components are present in trace amounts. A second picture is interpreted only for Columbia Tacoma and shows essentially no increase in resolution (Table III).

The patterns in Fig. 2 for Michigan White, a blend of White Wheats grown in Michigan, are not typical of the White Wheat class, which do not follow a consistent pattern. The results in Table II indicate that the Semi-Dwarf variety resembles the winter flours in general contour. On the other hand, Brevor is more similar in distribution to the Hard Red Spring wheats, while Michigan White gives an intermediate distribution pattern. The interpretations for the second pictures (Table III) indicate considerable increase in resolution. Those interpreted as single components in the first picture resolve into additional components in the second picture. In Michigan White, components F and F′ appear in the second picture along with corresponding decreases in the amounts of components E and G which also appeared in the first picture. In the case of Semi-Dwarf, the increased resolution evident in components D′, E and G of the second picture is at the expense of components D, F and F′ in the first picture. In Brevor, the increased resolution in the second picture is manifested in component E.

Omar was the only Club wheat studied. The interpretation of the first picture (Table II) shows a resemblance in distribution to the Hard Red Spring class. No appreciable increase in resolution was experienced in the interpretation of the second picture (Table III).

The patterns in Fig. 2 and the results in Table II for T-mix Durum indicate a distribution different from that in the other classes studied. Components D and F′ seem to predominate equally. Additional components, D′ and G, appear in the second picture (Table III). Component F in the second picture replaces F′ in the first picture.

The results listed under Soft Red Winter wheats are for the determinations made on glutens from patent cake flours. All the values in Table II for the first picture are, in general, consistent. The results for glutens from bleached and unbleached flours (Cake flour 1) present no significant differences, and agree well with the analyses for the glutens of the other cake flour (Cake flour 2). The last three analyses (triplicate determinations on the same sample of gluten from Cake flour 2) demonstrate the reproducibility of the electrophoretic method in this instance. The values for the second picture in Table III reveal further resolution of components D′ and F′ for the gluten of Cake flour 2. In general, the appearance of the gluten from cake flour patterns is similar to, but not identical with, the patterns for Michigan White.

Acetic acid extracts of flour

The purpose in examining 0·01M-acetic acid extracts of the flours as well as the glutens for the various varieties of wheat was to demonstrate possible differences in the flours that might not appear in the gluten analyses. In most cases, the patterns for the 0·01M-acetic acid extracts of gluten could be superimposed on the patterns for the 0·01M-acetic acid extracts of flour. Inspection of the patterns in Fig. 1 for the Thatcher wheat shows that the intermediate components of the flour extracts are similar in contour at least to the main components of gluten, although the concentration of the total solids in the flour extracts are greater. There are, of course, more ' salt-soluble ' components (A, B, C, H′ and I) present in the flour extracts. While no patterns are presented for the other varieties, there is even more striking similarity between the gluten and flour patterns for the Hard Red Winter wheat class. Those varieties showing the most difference between the gluten and flour patterns are Semi-Dwarf, Brevor and Omar. Continuing the electrophoresis until the fast components left the field and the intermediate (gluten) components extended throughout the field afforded added resolution in most cases.

The quantitative interpretation of the first pictures is presented in Table IV. The percentage distribution and mobility results for 14 components are indicated, but all components are not present in every flour. Component D is the predominating component in most varieties. The component having zero mobility is presumably largely polysaccharide. The general distribution of components from the standpoint of mobility is fairly consistent for the Hard Red Spring class. Differences in the percentage distribution are apparent, especially for component D. Willet has a higher content of D than the other varieties. With further resolution into more components as indicated by the interpretations of the second pictures (Table V), the distribution is somewhat altered. Component D is higher for Lee and Thatcher than for Willet or Selkirk wheats. Willet has slightly less of components E and H′, but more of F than others of its group.

There is also excellent consistency within the Hard Red Winter wheat class for the interpretation of the first pictures (Table IV). The consistency is good for the second picture with

Table IV

Electrophoretic data for flour extracts

(first picture)

Phosphate buffer, pH 3·0, $\Gamma/2$ 0·05, K = 2500–2680 μmhos/cm., 6·43–6·88 V/cm.

Variety	A μ	A %	B μ	B %	B′ μ	B′ %	C μ	C %	C′ μ	C′ %	C″ μ	C″ %	D μ	D %	E μ	E %	G μ	G %	H μ	H %	H′ μ	H′ μ	I μ	I %
Hard Red Spring wheats																								
Willet					7·55	4	6·48	2	5·60	1	4·93	2	3·66	49	2·34	13	1·83	7	1·06	2	0·59	3	0	17
Selkirk	9·12	1			7·97	6	6·55	4	5·13	4			3·63	31	2·60	18	1·89	8	1·33	3	0·65	5	0	20
Lee			8·09	3	7·06	3	6·18	3			4·97	4	3·65	42	2·64	17	1·99	8	1·37	3	0·58	5	0	12
Thatcher	9·27	1	8·02	4			6·47	3	5·01	4			3·71	38	2·67	19	1·92	5	1·34	2	0·67	4	0	20
Hard Red Winter wheats																								
Itana	8·78	1			7·52	3	6·58	2	5·54	2	4·70	2	3·42	33	2·57	14	1·92	11	1·13	4			0	28
Wichita	9·37	2			7·93	7	6·59	4	5·30	3			3·60	28	2·68	12	2·00	11	1·13	2			0	31
Bison	9·20	1			7·90	6	6·54	4	5·32	2			3·72	33	2·55	13	1·94	11	1·16	2			0	28
Columbia Tacoma	8·91	2			7·48	8	6·26	3	5·37	2	4·67	4	3·46	29	2·51	12	1·77	8	1·21	2			0	30
White wheats																								
Michigan	9·07	2			7·73	8	6·58	3	5·60	1	4·93	2	3·68	27	2·74	15	1·88	11	1·03	3			0	28
Semi-Dwarf			8·72	2	7·54	10	6·45	3	5·48	2	4·64	2	3·97*	3*	2·60	12	2·04	10	1·21	2	0·59	4	0	29
Brevor	9·03	1			7·77	7	6·68	4	5·58	3	4·75	3	3·97†	6†	2·72	15	2·20	11	1·29	4	0·67	4	0	29
Club wheat																								
Omar	9·18	1			7·79	7	6·40	2	5·20	2	4·43	2	3·48	20	2·66	19	2·08‡	6‡	1·18	2	0·74	2	0	34
Omar	9·02	2			7·61	8	6·21	4			4·76	3	3·53	19	2·72	13	2·14§	5§	1·26	2	0·72	5	0	37
Durum wheat																								
T-mix Durum			8·78	2	7·36	3	6·50	3	5·63	2	4·81	2	3·67	25	2·81	12	2·14	19	1·24	2			0	30
Soft Red Winter wheats																								
Cake flour 1 unbleached			8·83	3	7·44	9	6·17	4	5·12	5			3·44	29	2·51	12	1·86	8	1·30	1	0·74	3	0	26
Cake flour 1 bleached			8·68	2	7·66	9	6·27	3	5·02	4			3·62	30	2·64	13	1·93	8	1·20	1	0·68	3	0	27
Cake flour 2			8·91	1	7·65	8	6·00	4			4·94	4	3·68	29	2·62	15	1·86	8	1·22	1	0·73	4	0	26

* Peak D′ μ 3·27, 21% † Peak D′ μ 3·28, 13% ‡ Peak G′ μ 1·64, 3% § Peak G′ μ 1·69, 2%

Table V

Electrophoretic data for flour extracts

(As Table IV, second picture)

Peak	C′		C″		D		D′		E		F		G		G′		H		H′		I		A–C
Variety	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	%
Hard Red Spring wheats																							
Willet	5·60	1	4·93	2	3·79	26	3·33	13	2·83	8	2·34	14	1·79	7			1·11	2	0·51	3	0	17	7
Selkirk	5·13	2			3·73	23	3·15	13	2·72	12	2·32	10	1·88	6			1·24	2	0·63	5	0	20	7
Lee			4·97	3	3·76	35	3·12	11	2·74	11	2·35	8	1·98	6			1·34	1	0·62	5	0	13	7
Thatcher	5·01	4	4·77	3	3·88	32	3·35	7	2·91	11	2·47	7	2·06	5			1·41	2	0·65	6	0	15	8
Hard Red Winter wheats																							
Wichita	5·30	3	4·12	3	3·57	19	3·08	6	2·71	8	2·41	5	2·08	8	1·43	1			0·62	4	0	29	14
Bison	5·32	2	4·47	3	3·65	26	3·02	6	2·70	7	2·40	5	2·05	10	1·40	2			0·55	6	0	22	11
Columbia Tacoma	5·37	2	4·42	7	3·56	24	3·01	5	2·69	8	2·31	3	1·94	8			1·20	2	0·53	4	0	24	13
White wheats																							
Michigan	5·26	4	4·29	2	3·62	21	3·09	9	2·75	11	2·32	4	1·88	7			1·16	2	0·60	4	0	24	13
Semi-Dwarf	5·48	3	4·64	2	3·68	6	3·26	12	2·73	10	2·36	5	2·08	7	1·44	2			0·56	5	0	29	16
			4·97	3																			
Brevor	5·58	3	4·75	3	3·66	5	3·27	11	2·81	11	2·49	6	2·18	10	1·47	3	0·84	4	0·45	4	0	24	12
			4·00	4																			
Club wheat																							
Omar	4·23	5	3·66	5	3·34	11	2·94	5	2·58	10	2·26	4	2·02	4	1·69	2	1·10	2	0·63	2	0	36	14
Omar	4·02	2	3·72	3	3·44	7	3·04	5	2·70	8	2·34	6	2·02	4	1·74	2	1·18	6	0·61	5	0	35	17
Soft Red Winter wheats																							
Cake flour 1 unbleached	5·12	5	4·18	3	3·42	21	2·93	6	2·57	8	2·19	6	1·78	5			1·17	1	0·61	3	0	26	16
Cake flour 1 bleached	5·02	2			3·61	25	3·01	9	2·62	9	2·28	7	1·89	8	1·20	1	0·71	2	0·36	5	0	24	8
Cake flour 2	4·94	4	4·21	3	3·55	21	2·94	7	2·53	8	2·16	4	1·83	5			1·30	1	0·56	5	0	23	19

added resolution into more components (Table V). The amounts of components D and I in the second picture for Wichita are different from the amounts for Bison and Columbia Tacoma wheats.

As in the case of the glutens, the analyses of flour suspensions within the White wheat class present little consistency for the first picture (Table IV). Inspection of the results for the second picture reveals considerably more resolution of the gluten components and considerably more consistency between Brevor and Semi-Dwarf (Table V). Reproducibility between the two analyses for Omar of the Club class was satisfactory in the interpretation of the first pictures (Table IV). Resolution was less sharp than in the case of the gluten. The results for the second picture (Table V) reveal greater resolution.

Since only one picture was interpreted for T-mix Durum flour, the values are presented in Table IV. Components D and G predominated, but in different proportions from those (components D and F) appearing in gluten, Table II.

The results for the first pictures of the cake flours (Soft Red Winter wheat class) present a fairly consistent delineation (Table IV). No outstanding differences exist between the bleached and unbleached flours, notwithstanding the greater resolution of the second pictures (Table V).

Sodium chloride extracts of flour

The flour samples shown in Table I were extracted with 0·1M-sodium chloride, as described,[10] and the extracts analysed in both veronal and borate buffers. Reproducibility of the electrophoretic technique in these buffers was illustrated previously.[10]

(i) *Analyses in veronal buffer*

Electrophoretic analyses in veronal buffer, pH 8·6, $\Gamma/2$ 0·1, yielded patterns which in overall contour were fairly similar, with good enantiography. The Thatcher flour extract (Fig. 3) represents a typical analysis. The sharp peak (component 9) is particularly characteristic. The appearance of a distinct component 8 is also noteworthy. With the exception of the Willet extract (Fig. 3) this peak was observed in the eight Hard Red Spring and Hard Red Winter wheats investigated. It was also apparent in the Semi-Dwarf extract (a White wheat) and in the Omar extract (a Club wheat). As discussed previously[10] this component was not

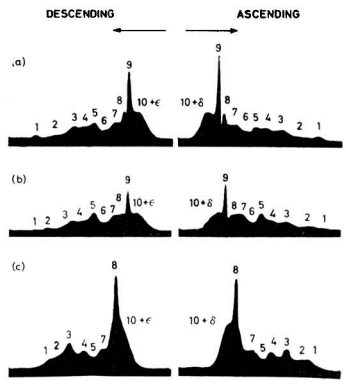

FIG. 3.—*Electrophoretic patterns of* 0·1M-*sodium chloride extracts of flours analysed in veronal buffer* (pH 8·6, *I*/2 0·1)

(*a*) Thatcher: 1%, 201·9 min., 35°
(*b*) Willet: 0·8%, 203·0 min., 35°
(*c*) T-mix Durum: 1·1%, 172·8 min., 45°

distinct in a Soft wheat patent flour until defatted according to the procedure of Mecham & Mohammad.[12] The patterns of the T-mix Durum flour extract are also shown in Fig. 3 to illustrate its unique distribution of electrophoretic components.

Electrophoretic mobilities and percentage distribution of the components resolved from these extracts are given in Table VI. It should again be stressed that interpretation of the patterns is somewhat arbitrary and that an effort to be consistent has been made. At least ten components, including the anomaly, were resolved in the veronal analyses. Little variation in component distribution was noted within the Hard Red Spring and the Hard Red Winter classes. The Michigan White flour differed in its distribution of components 8 and 10 compared to the other two White wheats.

The most striking differences in the distribution of the electrophoretic components were noted among the wheat classes rather than between the individual wheats. By arranging the

Table VI

Distribution data for sodium chloride extracts of various varieties of wheat flour

(Veronal buffer, pH 8·6, *I*/2 0·1. Field strength, 6·9-7·2 V/cm. Current 16 ma.)

Variety	*Components*																		*Component groups*		
	1		2		3		4		5		6		7		8		9		10		1+2	3-7	8-10
	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%			
Hard Red Spring wheats																							
Willet	6·12	1	4·94	4	3·93	9	3·24	9	2·62	12	1·96	5	1·43	10	0·89	7	0·56	15	0	28	5	45	50
Selkirk	6·18	1	5·12	4	4·06	9	3·37	9	2·69	10	2·04	5	1·50	12	0·97	9	0·60	17	0	25	5	44	51
Lee	6·09	1	4·88	3	3·87	8	3·27	7	2·66	10	1·99	4	1·49	11	1·01	9	0·64	25	0	22	4	40	56
Thatcher	6·07	1	4·89	4	3·95	9	3·30	8	2·64	10	1·96	5	1·46	11	0·99	7	0·65	20	0	25	5	43	52
																			Average		5±0·5	43±2	52±2
Hard Red Winter wheats																							
Itana			5·12	1	3·78	7	3·13	4	2·55	7	1·97	3	1·44	9	0·93	7	0·61	24	0	38	1	30	69
Wichita	5·27	1	4·34	2	3·75	7	3·22	4	2·60	8	1·95	3	1·43	7	0·93	11	0·56	26	0	31	1	31	68
Bison	5·40	2	4·62	1	3·91	7	3·28	5	2·64	9	2·04	3	1·50	8	0·98	9	0·64	21	0	35	2	33	65
Columbia Tacoma	5·13	1	4·28	1	3·84	5	3·21	4	2·57	8	1·96	3	1·38	8	0·85	11	0·54	25	0	34	1	29	70
																			Average		1±0·5	31±1·5	68±2
White wheats																							
Michigan White	5·51	1	4·72	1	4·08	8	3·34	5	2·65	8	2·10	4	1·48	13	0·90	10	0·58	20	0	30	2	38	60
Semi-Dwarf	6·30	1	5·30	3	4·14	11	3·41	7	2·74	10	2·10	5	1·56	11	0·97	6	0·58	23	0	23	4	44	52
Brevor	—	1	5·36	1	3·93	10	3·21	6	2·65	8	1·94	5	1·45	11	1·03	6	0·61	29	0	23	1	40	58
																			Average		2±1	41±3	57±3
Club wheat																							
Omar	5·27	1	4·48	1	3·94	6	3·23	3	2·63	7	2·05	2	1·49	9	0·94	6	0·58	27	0	38	2	28	70
Durum wheat																							
T-mix Durum	5·87 5·32	1 3	4·77	3	4·07	13	3·04	10	2·35	3			1·66	10	0·83	37			0	20	7	36	57
Soft Red Winter wheats																							
Cake flour 1 unbleached	5·74	2	4·76	2	4·16	6	3·44	6	2·78	11	2·08	3	1·55	11	0·98	6	0·62	25	0	28	4	37	59
Cake flour 1 bleached	—	1	—	1	4·11	4	3·33	7	2·70	11	2·11	4	1·53	13	1·00	9	0·67	27	0	23	2	39	59

components into three groups, that is, components 1 and 2, components 3 to 7, and components 8 to 10 (Table VI), the following observations were made:

The slower moving material (components 8–10) was present in largest amounts in the Hard Red Winter wheats (68%) and in the single Club wheat investigated (Omar) (70%), while the combined concentrations of components 3–7 were 31% and 28%, respectively. On the other hand, the Hard Red Spring wheats averaged 52% and 43%, respectively, for these two groups. The Durum flour was intermediate in distribution containing 57% and 36%, respectively, for these two groups. This flour had, however, considerable material (7%) in the fast mobility range (components 1 and 2). The three White wheats were not quite so consistent in their component distribution, but were similar to the Hard Red Spring wheats.

No significant differences in distribution were noted between a bleached and unbleached cake flour (Table VI).

(ii) *Analyses in borate buffer*

Whereas the sodium chloride extracts, when analysed in veronal buffer, produced similar patterns, analyses in borate buffer, pH 9·1, $\Gamma/2$ 0·1, yielded patterns which could be classified into four general groups, typical examples of which are shown in Fig. 4. The Thatcher flour extract (Fig. 4*a*) typifies the pattern observed for the Hard Red Spring wheat flours. The appearance of four major components (4, 6, 7 and 10) characterised this class. The Bison flour extract (Fig. 4*b*) typifies the Hard Red Winter flours. Enantiography was poorer for this class, suggesting component interaction during the electrophoretic analysis. The spiked peak evident on the ascending side (component 8) was common to this class. It appears that two of the components observed in Thatcher (components 6 and 7) have further resolved in Bison to give components 5 to 8. The White wheat flours also produced characteristic electrophoretic patterns but were not consistent as a class. Michigan White, shown in Fig. 4*c*, is somewhat typical of the White wheat flour extracts. Fig. 4*d* illustrates the unique distribution of the T-mix Durum flour extract.

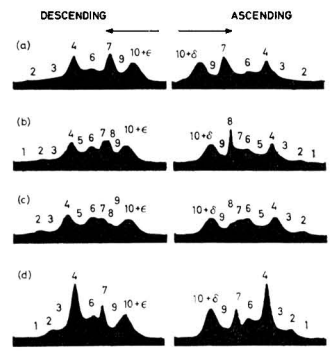

FIG. 4.—*Electrophoretic patterns of* 0·1M-*sodium chloride extracts of flour analysed in borate buffer* (pH 9·1, $\Gamma/2$ 0·1)

Diaphragm angle, 35°

(*a*) Thatcher: 0·85%, 244·9 min.
(*b*) Bison: 0·6%, 222·8 min.
(*c*) Michigan: 0·7%, 234·9 min.
(*d*) T-mix Durum: 1·0%, 201·5 min.

Component distribution and mobility data for the various wheat samples analysed in borate buffer are summarised in Table VII. Here, grouping the various components as was done with the veronal analyses showed no differences among the classes. Certain peculiarities were observed, however, among the individual flours. For example, the Willet flour extract contained less material of zero mobility than the other Hard Red Spring wheats. This flour extract also showed a rather diffuse spread-out pattern compared with the sharper peaks noted for Thatcher flour indicating possible distributional differences. Columbia Tacoma was low in component 7, compared with the other wheats in this class. Michigan White was lower in component 8, and slightly higher in component 4, giving it a somewhat different overall distribution pattern from the other White wheat flours. Again, as with the veronal analyses, no significant differences were noted between the bleached and unbleached cake flour.

Table VII

Distribution data for sodium chloride extracts of various varieties of wheat flour

(Borate buffer, pH 9·1, $\Gamma/2$ 0·1. Field strength, 6·4–6·8 V/cm. Current, 16 ma.)

Variety	1		2		3		4		5		6		7		8		9		10	
	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%	μ	%
Hard Red Spring wheats																				
Willet			5·37	3	4·47	3	3·54	25			2·26	20	1·45	24			0·78	6	0	19
Selkirk			5·26	1	4·26	5	3·54	20	2·60	18			1·50	25			0·80	6	0	25
Lee			5·76	2	4·44	5	3·44	23			2·34	17	1·41	21			0·74	7	0	25
Thatcher			5·61	2	4·44	5	3·41	24			2·32	17	1·34	24			0·69	4	0	24
Hard Red Winter wheats																				
Itana			5·11	4	4·07	3	3·29	18	2·66	7	2·06	12	1·41	13	1·13	12	0·76	4	0	27
Wichita			5·29	2	4·22	3	3·40	22	2·65	10	2·04	14	1·45	12	1·07	10	0·63	3	0	24
Bison	6·16	1	5·20	3	4·29	4	3·39	17	2·77	7	2·17	15	1·55	9	1·13	13	0·68	4	0	27
Columbia Tacoma			5·21	1	4·30	5	3·27	21	2·62	7	2·08	16	1·55	6	1·14	15	0·70	6	0	22
White wheats																				
Michigan White			5·21	4	4·48	2	3·70	23	2·88	9	2·21	18	1·59	9	1·12	7	0·64	4	0	24
Semi-Dwarf			5·02	5	4·16	4	3·43	19	2·71	9	2·07	15	1·48	11	1·08	11	0·71	6	0	20
Brevor			5·24	5	4·32	3	3·47	16	2·79	7	2·16	17	1·64	9	1·18	13	0·73	4	0	26
Club wheat																				
Omar			4·67	4	3·98	3	3·37	12	2·78	5	2·04	25	1·50	14	1·07	8	0·66	3	0	26
Durum																				
T-mix Durum	6·06	1	5·19	4	4·58	3	3·59	35			2·41	19	1·68	13			0·97	3	0	22
Soft Red Winter wheat																				
Cake flour 1 unbleached	5·74	1	4·70	3	4·17	3	3·51	14	2·88	6	2·32	16	1·39	27			0·71	6	0	24
Cake flour 1 bleached					4·28	5	3·71	13	3·12	8	2·34	19	1·56	26			0·78	6	0	23

Conclusions

Moving boundary electrophoresis has great potential for studying the proteins of the wheat kernel. This survey demonstrates that differences among the classes of wheats are manifested in the electrophoretic analyses of the ' salt-soluble ' and gluten proteins. To be specific, the Hard Red Spring, the Hard Red Winter and the Durum wheats show electrophoretic patterns which are characteristic of their respective classes, but the analyses of the White wheats were less consistent as a class. A small but definite difference in the contour of the electrophoretic patterns was observed in the ' salt-soluble ' proteins of Willet wheat as analysed in veronal buffer when compared with the other members of the Hard Red Spring wheat class. Small differences were also observed in the gluten proteins of Willet flour.

The interpretation of electrophoretic patterns at a second (longer) time interval furnished additional information because of the increased resolution of the slower components of the gluten and flour extracts with phosphate buffer.

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DRYING OF SEAWEEDS AND OTHER PLANTS. V.*—Through-circulation Drying of *Ascophyllum nodosum* in a Semi-continuous Dryer†

By J. H. MERRITT‡ and E. GORDON YOUNG

The drying of the common rockweed, *Ascophyllum nodosum*, has been studied on a semi-commercial scale by through-circulation in a semi-continuous dryer of original design to determine optimum conditions. A feasible loading for fresh rockweed of 80% initial moisture content was approximately 6·0 lb./sq. ft./tray with air flow up to 80 lb. of dry air/min./sq. ft. At temperatures from 120° to 210° F heat consumptions of 1200–2000 B.Th.U./lb. of water evaporated were recorded. The yield of alginate was unaffected by the temperature employed, but discoloration of the powdered weed and of the extracted product occurred in direct proportion to it. The coefficient of viscosity of the alginate was approximately inversely proportional to the temperature of drying.

Introduction

There is little published information on the drying of seaweeds in the scientific literature. Previous studies have been made on the drying of rockweed and kelp with a batch dryer[1, 2] and recently on Irish moss[3] with the semi-continuous dryer used in the present series of experiments. Both dryers were built on a semi-commercial scale. Mitchell & Potts[4] found the optimum conditions for drying *A. nodosum* in a through-circulation laboratory dryer to be a bed depth of 2·0 lb. B.D.S. (bone dry solids)/sq. ft. with air mass flow of 10 lb. of dry air/min/sq. ft. for minced weed in single layers. Booth[5] has claimed that milled weed may be drum-dried with a heat consumption of <2000 B.Th.U./lb. of water evaporated but others have found that drying by through-circulation requires about 4000 B.Th.U./lb.

Rockweed serves as the primary source for the manufacture of sodium and other alginates in several countries because it is readily harvested and is very abundant in many localities. Unlike carrageenin from Irish moss, the alginates are resistant to degradation in neutral solution and are comparatively stable to heat. The more highly polymerised forms are slowly degraded above 120° F and decarboxylation of uronic acids occurs above 400° F.[6] The experiments described below were designed to test the efficacy of the dryer for rockweed and to provide data as a basis for the design of dryers of commercial size.

Experimental

The dryer (already described)[3] was 9 ft. long, 2 ft. wide and 7 ft. high. The air was heated by steam coils and circulated by a centrifugal fan. The seaweed passed through the dryer in six baskets on rollers. Rates of air mass flow of 50 and 80 lb. of dry air/min./sq. ft. were used without any recirculation.

Tests were conducted from August to September, 1960, and during July, 1961. After being harvested, the rockweed was either used the same day or it was stored in burlap bags immersed in the sea for not longer than 3 days before being dried. The plants received no preliminary treatment. Two lots of fresh weed were dried in warm air at about 90° F with an electric fan in the laboratory, to serve as controls.

The procedure of drying was the same as that used for Irish moss.[3] The initial moisture content was about 80% and the final about 16% by drying to constant weight at 105° C as shown in Table II.

Results

The optimum loading was found to be 1·2 lb. B.D.S./sq. ft. (~6·0 lb. of wet weed/sq. ft.) in each basket with a depth of approximately 6 in. This is the same as that for the batch dryer.[1]

The results at various temperatures and at two rates of air mass flow are shown in Table I. No tests were made with reheating or recirculation of air because approach to saturation of the air with water vapour occurred after the fifth basket.

* Part IV: *J. Sci. Fd Agric.*, 1961, **12**, 718 † Issued as N.R.C. paper

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Table I

Rate of output and heat consumption at various temperatures of operation

Control temp., °F	Output, lb. C.D.S.*/h./sq. ft.	Heat consumption, B.Th.U./lb. of water evaporated
120	8·1	1285
155	10·3	1710
180	13·8	1660
210	19·0	1525

Loading 1·2 lb. B.D.S./sq. ft./basket
Air mass flow 50 lb. dry air/min./sq. ft. at inlet
Recirculation zero
Make-up air temperature 70° F
Leakage of air from dryer approximately 14%

Control temp., °F	Output, lb. C.D.S.*/h./sq. ft.	Heat consumption, B.Th.U./lb. of water evaporated
120	10·0	1730
155	15·6	1890
180	19·0	2000
210	24·4	1990

Loading 1·2 lb. B.D.S./sq ft./basket
Air mass flow 80 lb. dry air/min./sq. ft. at inlet
Recirculation zero
Make-up air temperature 70° F
Leakage of air from dryer approximately 18%

* C.D.S. = commercial dry solids

In these and previous[3] results the amount of heat generated by friction between air and stock, etc., in the dryer, which has been estimated at 4000 B.Th.U./h./sq. ft. at air mass flow of 50 lb. of dry air/min./sq. ft. and 9000 B.Th.U./h./sq. ft. at 80 lb. of dry air/min./sq. ft., has not been included in the values of heat consumption. Included were the amount indicated by the steam flow meter, which accounted for the bulk of the total, and the calculated amount of heat added by the fan, based on the rate of air flow and temperature rise. All tests were made at ambient temperatures 65–85° F. The measured values of heat consumption were corrected to correspond to an ambient temperature of 70° F. Tests established that, within the accuracy of the instruments, heat input varied in direct proportion to the difference between the control temperature and the temperature of the make-up air.

The relationship of rate of air flow to drop in static pressure at a standard loading of 1·2 lb. B.D.S./sq. ft. in one basket corresponds closely to that for Irish moss[3] at a loading of 1·0 lb. B.D.S./sq. ft. This differs from previous results with a batch dryer[1] in which the pressure drops recorded for *A. nodosum* up to air mass flow 20 lb. of dry air/min./sq. ft. were lower than the present values. This is probably due to the effectiveness of the tops and bottoms of the baskets in confining the weed and thus preventing the formation of openings in wet and dry layers, particularly at the edges. In the batch dryer, the layers of weed rested on trays of expanded metal. Under the conditions of the tests, the resistance varied substantially as the first power of the velocity of air through the dryer. One might have expected a result in which the resistance varied as a power higher than one. These results suggest that a more rigorous investigation of pressure drop should be made.

Leakage of air from the dryer was 18% at maximum air flow as in previous tests.[3]

As it was desirable to know whether the temperature of drying had any effect on the extractability of the alginate, this was determined by extraction with 3% sodium carbonate at 40–50° C and estimation of the alginate in solution by the method of Percival & Ross[7] as modified by Jensen *et al.*[8] Sodium alginate of 97·6% purity was used as standard. The yields of sodium alginate extracted from the various samples of seaweed dried at different temperatures are shown in Table II. There does not appear to be any relationship with the drying temperature.

Sodium alginate was prepared from representative samples by the method of Vincent[9] but without any treatment to decolorise. The purity of these preparations was about 87% sodium alginate. Differences in coloration were observed from the cream of the control dried at 90° F to the dark brown of that dried at 210° F. The optical transmission of a 0·25% solution is shown numerically in Table II. This discoloration is probably due to the oxidation of phenolic compounds in *A. nodosum* as shown by Haug & Larsen.[10] The relative viscosities of these preparations, clarified if necessary by filtering through a sintered-glass funnel, were determined with Ubbelohde capillary viscometers on a solution of 0·25% w/w in acetate–chloride buffer of pH 5·35. The results, expressed in centipoises or as reduced specific viscosity, are shown in Table II, and no effect of temperature of drying is apparent. The values obtained were so surprisingly low that degradation in preparation was suspected as observed by Haug.[11]

Table II

Effect of drying at various temperatures on product

Lot number	Temp., °F	Time in dryer, min.	Final moisture, %	Yield of alginate as % of total solids	Optical transmission,* %	Viscosity† as cP	Viscosity† η_{sp}/c
A–3	86	—	17·29	24·2	76	0·934	0·21
A–9	86	—	14·81	23·6			
A–10	120	50	15·99	23·9	71	0·926	0·17
A–6	155	30	15·53	19·3	59	0·941	0·24
A–2	180	35	16·25	25·8	57	0·972	0·44
A–4	180	29	16·65	23·4			
A–12	210	26	16·45	21·2	44	0·934	0·21

* Solution of 0·25% alginate at 410 mμ vs solvent
† Solution of 0·25% alginate (corrected) in acetate chloride buffer at pH 5·35 and 25° c

An aliquot of 1 g. of the dry powdered plant was washed with 0·2N-hydrochloric acid at 25° for 3 min. to remove laminarin and fucoidin, extracted with 3% Na_2CO_3 at 40–50° c for 2 h. to dissolve the sodium alginate, and this extract clarified by centrifugation. The viscosity of the supernatant fluid was then determined in a Stormer viscometer and the concentration of alginate calculated from the known concentration in the sample. The results are shown in Table III. Intrinsic viscosities were calculated by applying the Baker–Philippoff formula which is moderately accurate as applied to solutions of alginate according to Haug.[11] This

Table III

Effect of drying at various temperatures on viscosity of extractable alginate

Lot no.	Temp., °F	Concn. of alginate %	Viscosity as cP	Viscosity η_{sp}/c	Viscosity $[\eta]$
2	180	0·22	2·28	7·04	5·6
3	86	0·21	4·42	19·2	15·8
6	155	0·16	1·41	3·54	2·9
9	86	0·20	2·22	7·47	6·0
10	120	0·21	1·83	5·17	4·2
12	210	0·18	1·38	3·02	2·4

formula states that $[\eta] = 8/c(\eta_{rel} - 1)$ where c is the concentration in g./100 ml. A definite relationship to temperature of drying is apparent although the viscosity of sample No. 6 is somewhat out of line. The values in Table III are only of relative significance because protein was present in the native or denatured state and its influence on the viscosity is unknown. However, the effect of raising the temperature of drying on the viscosity of the carbonate extract is apparent.

Discussion

The conclusion to be drawn from these experiments is that the drying of rockweed is similar to the drying of Irish moss except that six passes of air through the rockweed, loaded at 1·2 lb. B.D.S./sq. ft., are required to ensure maximum evaporation without recirculation. A commercial dryer could thus be designed which would serve to dry either type of seaweed.

The greater stability of alginate to heat is notable in comparison with carrageenin when marked degradation was apparent at 155° F. However, alginate has again been shown to be degraded in the process of drying as shown by Haug.[11] It is thus obvious that the lowest feasible temperature for the drying of rockweed is desirable to maintain the natural viscosity of the alginate. Isolation of relatively pure sodium alginate by conventional methods appears to degrade it severely.

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SOIL SALINITY STUDIES. I.—Effect of Calcium Sulphate on the Correlation between Plant Growth and Electrical Conductivity of Soil Extracts

By G. W. WINSOR, J. N. DAVIES and D. M. MASSEY

The relation between the growth of lettuce and soil salinity (estimated by seven methods) was studied in the presence of varying levels of calcium sulphate. Lettuce were grown in pots receiving five levels of a mixture of potassium nitrate and diammonium hydrogen phosphate and four levels of calcium sulphate in factorial combination.

Soil salinity was measured by the electrical conductivity of displaced soil solutions, saturation extracts, and soil suspensions prepared at water/soil (air-dry) ratios of 1 : 1, 2·5 : 1 and 5 : 1. Direct measurements of electrical conductivity were also made in the saturation pastes, and a tentative procedure involving saturation of all 2·5 : 1 soil suspensions with calcium sulphate was examined.

Correlation between fresh weights of lettuce and conductivity measurements at water/soil ratios of 5 : 1 and 2·5 : 1 was unsatisfactory, but was better at a ratio of 1 : 1. Highly significant correlations of plant weight with measurements in saturation extracts and displaced soil solutions were obtained. Direct measurements in the saturation pastes gave correlations almost identical with those for filtrates from the pastes, but the effect of differences in soil texture on this relationship remains to be examined. The highest correlations of all were obtained in soil suspensions saturated with calcium sulphate.

Introduction

Soil salinity is a natural problem in arid regions, but under humid conditions is usually man-made, arising from excessive application of fertilisers. Saline soils have been studied intensively at the United States Salinity Laboratory,[1] the electrical conductivity of the saturation extract being recommended as a general method for assessing salinity. The special advantage claimed for this procedure is that the saturation percentage is directly related to the field moisture range. Soil texture is thus automatically taken into account, and this in turn simplifies the relationship between electrical conductivity and plant growth. The influence of soil texture is not included in the present investigation, the various salinities being produced by adding fertiliser to one soil type. A further advantage of the saturation extract procedure,

however, is the low water/soil ratio used. This reduces interference from sparingly soluble salts which are dissolved to an increasing extent with the ratio of water to soil used in the salinity measurement. Despite the soundness of these recommendations, conductivity measurements in soil suspensions at higher ratios of water to soil are undoubtedly far more convenient for advisory purposes. The saturation extract has not been universally adopted in America, whilst in this country almost all routine salinity measurements have for many years been made at a water/soil ratio of 2·5 : 1 or even at higher ratios. Whilst it is likely that a more enlightened use of fertilisers under glass is already reducing the occurrence of excessive salinity, many old glasshouse soils contain more than enough calcium sulphate to saturate the soil solution. Adding water in preparing soil suspensions for conductivity measurements must therefore lead in many instances to false results ; examples have been reported by Smith & Warren,[2] and by Butters.[3]

In this investigation was studied the effect of different levels of calcium sulphate on the growth of lettuce and on the conductivity results obtained by a range of methods. The work follows a preliminary investigation reported in 1956.[4]

Experimental

(a) *Glasshouse trials*

Two experiments are described in which lettuce were grown at five levels of fertiliser combined factorially with four levels of calcium sulphate. The fertiliser was composed of equal weights of diammonium hydrogen phosphate and potassium nitrate and the mixture had N 17·5%, P_2O_5 26·9% and K_2O 23·3% ; analytical grade salts were used. The percentages by weight of mixed fertiliser and of calcium sulphate in each experiment are given in the appropriate Tables.

The first three experiments in the series are not reported, as the test plants grew poorly. The soil used for Experiment IV was a steamed brick-earth top-soil, taken from under grass. For Experiment V the top 4 in. of soil, including the grass, were stacked and allowed to rot down before use. Because manganese toxicity occurred in the previous experiments this soil was not steamed before use. In both experiments the soil was mixed with peat (5 : 1 by volume) to improve the conditions for plant growth. Finely divided calcium carbonate was added at the rate of 0·2% throughout, with additional dressings equivalent to 10% of the weight of mixed fertiliser in each treatment.

Lettuce seedlings of varieties ' Webb's Wonderful ' (Experiment IV, April 1959) and ' Cheshunt 5B ' (Experiment V, September 1959) were grown singly in 6-in. clay pots, the treatments in both experiments being replicated fifteen times ; each replicate contained one pot of each of the twenty treatments, fully randomised.

The pots were watered individually as necessary to maintain growth with minimal leaching. No attempt was made to grow the lettuce to maturity, as watering became difficult when the outer leaves covered the soil surface. The plants in Experiment IV were cut and weighed individually 26 days after planting. In Experiment V, seven replicates were harvested after 16 days and the remaining eight replicates after 27 days.

(b) *Measurement of soil salinity*

Immediately after the plants had been harvested, the soil from individual pots of each treatment was bulked. Samples were taken for displacement of soil solution at the moisture content prevailing at the end of the experiment. The moist soils were packed into tubes of 2-in. dia. to a depth of about 12 in. and the soil solution displaced with alcohol.

Further soil samples were air-dried for subsequent examination. Saturation paste extracts were made by the procedure of the United States Salinity Laboratory.[1] Soil suspensions were prepared from air-dry soil at water/soil ratios of 1 : 1, 2·5 : 1 and 5 : 1. After the solutions had been shaken and set aside for consecutive periods of 30 min. their electrical conductivities were measured at 20° with platinised platinum electrodes and an a.c. bridge (Mullard, Type E7566). The results were converted to specific conductivities (ohm^{-1} $cm.^{-1}$). In Tables III and V the results for 2·5 : 1 suspensions have been expressed in terms of pC as defined by

Whittles & Schofield-Palmer,[5] other results being given directly in millimhos (specific conductivity $\times 10^3$).

In addition to the accepted procedures, direct measurements of the electrical conductivities of the saturation pastes were made with an industrial-type conductivity cell with annular electrodes of carbon (Electronic Switchgear Ltd.). In order to eliminate variations in conductivity due to calcium sulphate in the soil, measurements were also made in 2·5 : 1 water/soil suspensions to which excess calcium sulphate ($CaSO_4,2H_2O$ 0·5 g. per 100 ml.) was added before shaking.

Results

(a) *Experiment IV*

Within a week of transplanting, many of the plants having the highest level of fertiliser (1%) were dying; further losses occurred as the experiment continued, particularly at the 0·8% and 1% levels. The numbers of plants lost before harvesting, shown in Table I, amounted to 14·3% of the total number of plants in the experiment. This partial crop failure was attributed to the use of steamed soil; partial sterilisation was therefore omitted in subsequent experiments. As the losses were closely associated with the levels of fertiliser application, no correction was made for missing plants. The mean weights of lettuce for each treatment in Table II are the total weights of plants surviving at harvest divided by the original number planted (15).

Table I

Numbers of plants dead before harvesting (after 26 days) in relation to soil salinity (Experiment IV, initially 15 plants per treatment)

$CaSO_4,2H_2O$ added, %	Fertiliser added, %					Total
	0·2	0·4	0·6	0·8	1·0	
0	0	0	0	1	4	5
0·2	0	1	4	9	9	23
0·5	0	0	2	3	2	7
1·0	0	0	0	4	4	8
Total	0	1	6	17	19	

Table II

Mean weights (g.) *of lettuce plants grown for 26 days with different combinations of mixed fertiliser and calcium sulphate* (Experiment IV)

$CaSO_4,2H_2O$ added, %	Fertiliser added, %					Mean
	0·2	0·4	0·6	0·8	1·0	
0	34·6	23·7	9·5	6·0	2·4	15·2
0·2	32·2	20·3	8·0	2·2	0·2	12·6
0·5	32·5	17·4	7·2	1·2	1·6	12·0
1·0	36·1	22·9	14·1	1·6	0·9	15·1
Mean	33·9	21·1	9·7	2·8	1·3	

L.S.D. (P = 0·05) between calcium sulphate levels : ±1·77
,, ,, ,, fertiliser levels : ±1·98
,, ,, ,, individual values in the body of the table : ±3·95

The fresh weights of the lettuce plants decreased progressively with increasing salinity, each increment of fertiliser producing a significant depression in growth up to the 0·8% level. Significant differences also occurred between mean lettuce weights at the various levels of calcium sulphate, but there was no consistent response to the treatments, and the range of variation was relatively small in comparison with the results for fertiliser levels.

The conductivity results from the six methods are too extensive to reproduce in full, but the pC values as determined in 2·5 : 1 suspensions, given in Table III, indicate the levels of salinity according to the procedure most widely used in this country. Data for the saturation extracts and displaced soil solutions are also included in the Table, each value being the mean for four levels of calcium sulphate as this had relatively little effect on these determinations.

Table III

Soil salinity at the end of Experiment IV, as determined in (a) 2·5 : 1 aqueous suspensions, expressed as pC values, (b) saturation paste filtrates and displaced soil solutions, expressed in millimhos per cm. (means for four levels of calcium sulphate)

(*a*) pC values in 2·5 : 1 suspensions

$CaSO_4.2H_2O$ added, %	Fertiliser added, %					Mean
	0·2	0·4	0·6	0·8	1·0	
0	3·18	2·89	2·78	2·80	2·77	2·88
0·2	2·86	2·79	2·76	2·73	2·73	2·77
0·5	2·65	2·62	2·64	2·59	2·58	2·62
1·0	2·65	2·59	2·55	2·52	2·49	2·56
Mean	2·84	2·72	2·68	2·66	2·64	

(*b*) Mean conductivity (millimhos) of saturation extracts and displaced soil solutions

Saturation extract	3·66	5·88	6·97	7·45	8·35
Soil solution	6·58	13·56	17·72	18·17	20·00

The results for the 2·5 : 1 aqueous suspensions show that this test was influenced at least as much by the levels of calcium sulphate as by the soluble fertiliser, whereas the lettuce plants (Table II) were affected mainly by the latter alone.

(b) *Experiment V*

The lettuce in this experiment grew satisfactorily and, in contrast to the preceding trials, no plants died before harvest at any level of salinity. Seven replications of the treatments were harvested 16 days after transplanting when the approximate diameters of the plants were 7, 6½, 5¾, 5½ and 4½ in. at 0·15, 0·3, 0·5, 0·7 and 0·9% of added fertiliser respectively. The mean fresh weights of the lettuce are in Table IV. There was a highly significant decrease in weight with increasing salinity. Significant differences in fresh weight also occurred between lettuce grown at different levels of calcium sulphate, which seemed to benefit growth.

The remaining eight replications were harvested 27 days after transplanting, at which stage the lettuce grown at levels up to 0·5% of fertiliser were of about 9 in. diameter and were just beginning to heart. At the highest level of added fertiliser (0·9%) calcium sulphate increased plant size, the diameters being approximately 6, 7, 7½ and 8 in. at 0, 0·25, 0·5 and 1·0% respectively. The mean weights of lettuce (Table IV) again decreased progressively with increasing salinity. The effect of calcium sulphate on fresh weight was small and not statistically significant.

The pC values determined in 2·5 : 1 aqueous suspensions are given in Table V ; the results are for samples taken at the first harvest. Calcium sulphate had a far greater effect on the conventional conductivity test than on the lettuce plants. Furthermore, the growth of the lettuce up to the first harvest actually improved when the increase in conductivity (2·5 : 1 suspensions) was due to calcium sulphate rather than to fertiliser. Mean data for the saturation extracts and displaced soil solutions are also in Table V.

(c) *Correlation between growth and salinity as measured by various procedures*

Correlation coefficients relating mean fresh weight of lettuce and specific electrical conductivity measured under various conditions are given in Table VI. Each correlation coefficient is based on 20 soil treatments in which five levels of fertiliser were combined with four levels of calcium sulphate. The column headed ' Preliminary Trial ' is reproduced from an earlier report.[4]

Disregarding the results for direct measurements in the saturation pastes and for 2·5 : 1 suspensions with calcium sulphate added, the remaining five tests showed increasing correlations down each column of Table VI as the amount of water added for the conductivity measurement decreased. At a water/soil ratio of 5 : 1 only one out of the seven correlation coefficients was significant at $P = 0.05$. At 2·5 : 1, four out of seven correlation coefficients were significant, but two of these only at the 5% level. The two highest correlations at 2·5 : 1, and the only significant correlation at 5 : 1, were obtained when fresh weight was correlated with the initial

Table IV

Mean weights (g.) *of lettuce plants grown for* (*a*) 16 *days and* (*b*) 27 *days, with different combinations of mixed fertiliser and calcium sulphate* (Experiment V)

(*a*) Harvested after 16 days (7 replicates)

$CaSO_4,2H_2O$ added, %	Fertiliser added, %					Mean
	0·15	0·3	0·5	0·7	0·9	
0	6·9	6·9	6·7	4·3	2·2	5·4
0·25	7·5	8·6	5·9	4·2	3·9	6·0
0·5	8·7	7·2	5·9	4·2	3·5	5·9
1·0	8·9	7·4	7·6	5·5	3·3	6·5
Mean	8·0	7·5	6·5	4·6	3·2	

L.S.D. (P = 0·05) between calcium sulphate levels : ±0·38
,, ,, ,, fertiliser levels : ±0·43
,, ,, ,, individual values in the body of the table : ±0·85

(*b*) Harvested after 27 days (8 replicates)

$CaSO_4,2H_2O$ added, %	Fertiliser added, %					Mean
	0·15	0·3	0·5	0·7	0·9	
0	37·5	34·7	38·4	24·3	13·2	29·6
0·25	37·6	38·0	35·1	22·1	16·2	29·8
0·5	39·7	38·9	33·0	22·7	17·0	30·3
1·0	41·7	35·2	35·6	23·2	18·2	30·8
Mean	39·1	36·7	35·5	23·1	16·2	

L.S.D. (P = 0·05) between calcium sulphate levels : ±2·27
,, ,, ,, fertiliser levels : ±2·53
,, ,, ,, individual values in the body of the table : ±5·07

Table V

Soil salinity in Experiment V after cropping for 16 *days, as determined in* (*a*) 2·5 : 1 *aqueous suspensions, expressed as pC values,* (*b*) *saturation paste filtrates and displaced soil solutions, expressed in millimhos per cm.* (means for four levels of calcium sulphate)

(*a*) pC values in 2·5 : 1 suspensions

$CaSO_4,2H_2O$ added, %	Fertiliser added, %					Mean
	0·15	0·3	0·5	0·7	0·9	
0	3·13	2·97	2·84	2·74	2·77	2·89
0·25	2·82	2·72	2·64	2·61	2·65	2·69
0·5	2·66	2·62	2·55	2·50	2·50	2·57
1·0	2·65	2·60	2·54	2·46	2·42	2·53
Mean	2·82	2·73	2·64	2·58	2·57	

(*b*) Mean conductivity (millimhos) of saturation extracts and displaced soil solutions

Saturation extract	3·71	5·37	6·84	8·36	8·66
Soil solution	6·11	10·36	15·21	20·66	21·53

rather than the final salinity of the soils. This may be due to losses of soluble salts by leaching during the growing period, despite the care with which the pots were watered. The full conductivity results (not reproduced here) indicate some decrease in salinity as the experiments proceeded ; other factors possibly contributing to this decrease were absorption by the walls of the pots, uptake by the plants and absorption or fixation in the soil. The 1 : 1 water/soil suspensions always showed a significant correlation with plant growth, but the correlation coefficients were not as high as those for the saturation extracts and displaced soil solutions. Including sufficient calcium sulphate to saturate the 2·5 : 1 water/soil suspensions led to excellent correlations between growth and salinity measurements, the correlation coefficients frequently being higher than those for both the saturation extracts and displaced soil solutions.

Discussion

The results for fresh weights of lettuce in Tables II and IV show a very marked effect of salinity caused by soluble fertiliser. By comparison, the sparingly soluble calcium sulphate

Table VI

Correlations between mean fresh weight of lettuce (g.) *and soil salinity as measured by various procedures*

Each correlation coefficient is based on 20 soil treatments

	Preliminary trial‡	Experiment IV	Experiment V				
Period of growth (days)†	29	26	16	16	27	27	27
Time of soil sampling†	29	26	0	16	0	16	27
5 : 1 water/soil suspension	0·30	0·21	0·39	0·09	0·53*	0·22	0·28
2·5 : 1 ,, ,, ,,	0·48*	0·43	0·62**	0·33	0·71***	0·43	0·51*
2·5 : 1 + calcium sulphate	0·93***	0·95***	0·92***	0·91***	0·96***	0·91***	0·94***
1 : 1 water/soil suspension	0·69***	0·73***	0·82***	0·58**	0·89***	0·66***	0·78***
Saturation paste	—	0·94***	0·90***	0·82***	0·95***	0·83***	0·86***
,, ,, extract	0·84***	0·94***	0·90***	0·80***	0·95***	0·82***	0·84***
Displaced soil solution	0·88***	0·96***	—	0·89***	—	0·90***	0·91***

† Days from transplanting
*, ** and *** denote statistical significance at P = 0·05, 0·01 and 0·001 respectively
‡ From reference [4]

had a relatively small effect on plant growth. Furthermore, the effects of calcium sulphate observed could hardly be due to soil salinity ; thus in Experiment IV, despite small but significant differences in mean fresh weight of the plants at different levels of calcium sulphate, there was no consistent trend and the difference between 0 and 1% of added calcium sulphate was negligible. In Experiment V, at the first date of sampling calcium sulphate benefited growth, and although the effect upon fresh weight was no longer significant at the second harvesting, it still showed in the size of the plants grown at high salinities.

Examination of Tables III (*a*) and V (*a*) shows how misleading the widely used technique of measurement in 2·5 : 1 soil suspensions can be when calcium sulphate is present. In Table III, for example, the pC value fell to 2·65 with 0·2% of soluble fertiliser and 1·0% of calcium sulphate. This value would normally indicate dangerous salinity, but the plants (Table II) grew well. Yet with 1% of soluble fertiliser and no added calcium sulphate, giving a pC value of 2·77 in the 2·5 : 1 suspensions, growth was reduced to only 7% of that found at pC 2·65 in the previous example.

Without a published survey of calcium sulphate in glasshouse soils, it is not possible to assess the frequency or magnitude of errors arising from using 2·5 : 1 water/soil suspensions for measuring soil salinity. Relatively few soils will contain as much calcium sulphate as was added at the highest level in these experiments, although such soils certainly exist. Some error must, however, be introduced into salinity determinations by adding water to any soil containing more calcium sulphate than would saturate the soil solution at normal moisture contents during crop growth ; no precise estimate can be given, since the solubility of calcium sulphate is influenced by the nature and concentration of other salts in solution. In the absence of mutual solubility effects, approximately 0·05% of calcium sulphate (as $CaSO_4$) in the soil would theoretically be sufficient to saturate the soil solution at a moisture content of 25% and 18°. This is relatively little calcium sulphate by glasshouse standards, and would have to be much exceeded before the error induced at higher water/soil ratios became of practical significance. Many glasshouse soils do, however, contain sufficient calcium sulphate to interfere markedly in salinity determinations at a water/soil ratio of 2·5 : 1, and this procedure cannot be regarded as satisfactory.

As expected, the conductivities of displaced soil solutions and saturation extracts were highly correlated with plant growth. Displacement with alcohol is both tedious and difficult, and would be unsuitable for routine application. The saturation extract procedure, though more rapid than displacement methods and applicable to air-dried soils, still requires individual attention to each soil ; considerable judgment is required in bringing each sample to the 'saturation' point. Direct measurement of the conductivities of the saturation pastes, avoiding difficult filtration, gave results which were no less closely correlated with plant growth than were those obtained in filtrates from the pastes. The effects of soil texture remain to be explored and all our results were obtained with one soil type.

None of the procedures tested, however time-consuming or theoretically attractive, gave results more closely correlated with plant growth than those obtained simply by adding excess calcium sulphate to saturate the 2·5 : 1 soil suspensions. With calcium sulphate thus eliminated as a variable, the correlation obtained would depend on experimental accuracy in the laboratory. There is no doubt that 2·5 : 1 suspensions of air-dry soil can be prepared not only more readily, but also more reproducibly, than either saturation extracts or displaced soil solutions. The range of experimental values is, however, restricted by saturation of all extracts with calcium sulphate and the conductivity measurements must be precise. The method also obscures the contribution to salinity made by calcium sulphate at concentrations up to saturation in the soil solution.

These results emphasise how calcium sulphate can complicate the assessment of salinity when using 2·5 : 1 soil suspensions, and also show the correlation between plant growth and salinity as measured by a range of techniques. Other work, including relationships between electrical conductivity measurements in various types of soil suspension and extract, will be reported subsequently.

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STUDIES IN FUNGITOXICITY. III.*—Fungitoxicity of Certain β-Nitrostyrenes and Related Compounds

By M. PIANKA

Several β-nitrostyrenes and related compounds have been tested for fungitoxicity to certain economically important parasitic fungi. The activity depended on ring substitution and was most marked when the ring substituent was a nitro group. Small alkyl groups of 1–5 C on the 2-carbon atom enhanced the activity of these compounds, but with 7 C activity began to decrease and was lost with 11 C.

Introduction

In Parts I[1] and II[2] were described the toxicities of certain carbocyanines (in which $n = 2$) and anilinovinyl quaternaries (in which $n = 1$) containing the conjugated system

$$—\overset{+}{N}R{:}\overset{|}{C}{\cdot}(CH{:}CH)_n{\cdot}N<$$

against the spores of *Venturia inaequalis* (Cooke) Wint.; *Botrytis cinerea* Pers.; and *Fusarium bulbigenum*, Cooke & Massee, var. *lycopersici* (Brushi) Wollenw.

* Part II: *J. Sci. Fd Agric.*, 1959, **10**, 385

This study was extended to nitrostyrenes,[3] of the general formula $ArCH{:}C(R){\cdot}NO_2$, in which an electron-attracting group is attached to the end of the conjugated chain. Also related compounds were tested for fungitoxicity.

The antifungal and antibacterial properties of compounds of this type have been described in a number of papers.[4–17]

Experimental

The compounds now tested are shown in Tables I and II. The syntheses of Compounds nos. 1, 2, 4, 7, 10, 11, 17, 20–24, 25, 27, 31, 32, 34, 35, 37–41 were by methods described in the literature.

Table I

Results of fungitoxicity tests with β-nitrostyrenes

No. of compound	Name of compound	M.p.	Reference	*Venturia*	*Botrytis*	*Fusarium*
				LD_{95} values, p.p.m.		
1	1-Phenyl-2-nitroethylene	57–58°	24	320	650	770
2	1-Phenyl-2-nitroprop-1-ene	64°	28	490	600	>1000
3	1-Phenyl-2-nitrobut-1-ene	(b.p. 100°/0·5 mm.)		560	>1000	>1000
4	1-(4-Methoxyphenyl)-2-nitroethylene	86–87°	20	65	70	70
5	1-(4-Methoxyphenyl)-2-nitroprop-1-ene	46–47°	28	120	285	253
6	1-(4-Methoxyphenyl)-2-nitrobut-1-ene	55–56°		44	260	145
7	1-(4-Chlorophenyl)-2-nitroethylene	111–112°	29	96	94	77
8	1-(4-Chlorophenyl)-2-nitroprop-1-ene	88–89°		80	150	80
9	1-(4-Chlorophenyl)-2-nitrobut-1-ene	76–76·5°		60	300	140
10	1-(4-Nitrophenyl)-2-nitroethylene	201–202°	18	25	150	50
11	1-(4-Nitrophenyl)-2-nitroprop-1-ene	114–115°	19	8	25	20
12	1-(4-Nitrophenyl)-2-nitrobut-1-ene	103·5–104·5°		9	23	30
13	1-(4-Nitrophenyl)-2-nitropent-1-ene	101·5–102·5°		12	12	28
14	1-(4-Nitrophenyl)-2-nitrohept-1-ene	67–68°		21	64	62
15	1-(4-Nitrophenyl)-2-nitronon-1-ene	61·5–62·5°		34	>1000	>1000
16	1-(4-Nitrophenyl)-2-nitrotridec-1-ene	64–65°		>1000	>1000	>1000
17	1-(4-Dimethylaminophenyl)-2-nitroethylene	182–183°	20	27	93	82
18	1-(4-Dimethylaminophenyl)-2-nitroprop-1-ene	123°		140	>1000	470
19	1-(4-Dimethylaminophenyl)-2-nitrobut-1-ene	88–89°		17	>1000	166
20	1-(3-Nitrophenyl)-2-nitroethylene	124·5–125·5°	20	85	74	100
21	1-(3,4-Dimethoxyphenyl)-2-nitroethylene	140°	30	16	46	66
22	1-(3,4-Methylenedioxyphenyl)-2-nitroethylene	160·5–161°	20	17	38	71
23	1-(2,4-Dichlorophenyl)-2-nitroethylene	116°	8	45	19	22
24	1-(4-Nitrophenyl)-2-nitro-2-ethoxycarbonylethylene	72–73°	31	130	110	36

1-Phenyl-2-nitrobut-1-ene (Compound no. 3)

Benzaldehyde (42·4 g.), 1-nitropropane (35·6 g.) and 33% w/v solution of ethylamine in ethanol (5·6 g.) were kept at 50–55° for 92 h. The water that formed (5·2 c.c.) was separated and the crude oil dried (sodium sulphate) and fractionated. A pale yellow oil, b.p. 100°/0·5 mm., n_D^{20} 1·5723, was obtained (Found: N, 7·5. $C_{10}H_{11}NO_2$ requires N, 7·5%).

1-(4-Methoxyphenyl)-2-nitrobut-1-ene (Compound no. 6)

Anisaldehyde (24·6 g.), 1-nitropropane (16·2 g.) and n-butylamine (1·5 g.) were kept in a stoppered flask for 14 days. Benzene (20 c.c.) was then added and the solution dried (sodium sulphate) and fractionated. The fraction boiling at 128–158°/0·4 mm. was collected. On

addition of alcohol a solid crystallised out and was separated by filtration. Large lemon-yellow crystals were obtained, m.p. 55–56°, first from carbon tetrachloride and then from ethyl acetate (Found: N, 6·3. $C_{11}H_{13}NO_3$ requires N, 6·8%).

1-(4-*Chlorophenyl*)-2-*nitroprop*-1-*ene* (Compound no. 8)

p-Chlorobenzaldehyde (21 g.), nitroethane (11·3 g.) and n-butylamine (1·1 g.) were kept in a stoppered flask for 14 days. On addition of some alcohol, a solid crystallised out and was separated by filtration. Large lemon-yellow crystals were obtained from alcohol, m.p. 88–89° (Found: N, 7·1. $C_9H_8ClNO_2$ requires N, 7·3%).

Table II

Results of fungitoxicity tests

No. of compound	Name of compound	M.p.	Reference	*Venturia* LD_{95} values, p.p.m.	*Botrytis* LD_{95} values, p.p.m.	*Fusarium* LD_{95} values, p.p.m.
	2-Furyl derivatives					
25	1-(2-Furyl)-2-nitroethylene	74–76°	32	>1000	>1000	>1000
26	1-(2-Furyl)-2-nitroprop-1-ene	51–52°		>1000	>1000	>1000
27	1-(5-Nitro-2-furyl)-2-nitroethylene	143–144°	33	33	35	28
	2-Thiophen derivatives					
28	1-(2-Thienyl)-2-nitroethylene	80–80·5°		440	>1000	520
29	1-(2-Thienyl)-2-nitroprop-1-ene	67·5°		445	>1000	245
30	1-(5-Nitro-2-thienyl)-2-nitroprop-1-ene	122·5–123°		22	97	26
	Miscellaneous compounds					
31	1-(4-Methoxyphenyl)-2-chloroethylene	34°	34	>1000	>1000	>1000
32	1-Methoxy-1,3-diphenyl-2,4 dinitrobutane	151–152°	25	3200	>1000	>1000
33	1-Methoxy-1,3-di-(4-nitrophenyl)-2,4-dinitrobutane	112–113°		210	>1000	>1000
34	1-Ethoxy-1,3-diphenyl-2,4-dinitrobutane	156°	25	1500	>1000	>1000
35	1-Methoxy-1-phenyl-2-bromo-2-nitropropane	51–53°	26	90	>1000	>1000
36	1-Methoxy-1-(4-nitrophenyl)-2-bromo-2-nitropropane	170·5–171·5°		30	>1000	>1000
37	2-Nitrobiphenyl	37°		>1000	>1000	>1000
38	4-Nitrobiphenyl	114°		>1000	>1000	>1000
39	4,2′-Dinitrobiphenyl	92°		>1000	>1000	>1000
40	2,2′-Dinitrobiphenyl	119–120°		>1000	>1000	>1000
41	Biphenyl	71°		>1000	>1000	>1000

1-(4-*Chlorophenyl*)-2-*nitrobut*-1-*ene* (Compound no. 9)

This was prepared in the same way as compound no. 8, but with 1-nitropropane (8·9 g.). Pale yellow needles were obtained from carbon tetrachloride and then from ethyl acetate, m.p. 76–76·5° (Found: N, 6·8. $C_{10}H_{10}ClNO_2$ requires N, 6·6%).

1-(4-*Nitrophenyl*)-2-*nitrobut*-1-*ene* (Compound no. 12)

Method A.—A modification of the method of Baker & Wilson[18] and Priebs[19] was used. 1-Phenyl-2-nitrobut-1-ene (44·2 g.) was added to fuming nitric acid (65 c.c.) with stirring for 2 h. below 0°. The mixture was stirred for a further hour below 0°, poured on to crushed ice (1·5 kg.) and water (500 c.c.) and kept stirred for 16 h. The precipitated yellow solid was separated by filtration, washed with water and then methanol and dried in air. The solid was recrystallised from alcohol, carbon tetrachloride, xylene and acetone in that order. Large lemon-yellow crystals were obtained, m.p. 103·5–104·5° (Found: N, 12·9. $C_{10}H_{10}N_2O_4$ requires N, 13·2%).

Method B.—A modification of the methods of Kamlet[20] and Kollonitsch & Vita[21] was used. *p*-Nitrobenzaldehyde (7·6 g.), 1-nitropropane (4·6 g.), n-caproic acid (0·2 g.), piperidine (0·4 c.c.), tributyl borate (11·6 g.) and benzene (100 c.c.) were heated with stirring in a flask to which a Dean & Stark trap was attached. The solution was concentrated, and the crude product that separated was crystallised from carbon tetrachloride and then from methanol, yielding

large lemon-coloured prisms, m.p. 102–103°. They gave no depression of m.p. with the product made by method A.

The 1-nitroalkanes used in the four preparations next described were synthesised from the appropriate alkyl bromides and silver nitrite by the method of Kornblum *et al.*[22]

1-(4-*Nitrophenyl*)-2-*nitropent*-1-*ene* (Compound no. 13)

p-Nitrobenzaldehyde (7·6 g.), 1-nitrobutane (5·2 g.), n-butylamine (0·3 g.) and ethanol (20 c.c.) were refluxed for 14 h. The solution was concentrated, and the crude product that separated on cooling was filtered off and recrystallised from carbon tetrachloride and then from methanol, yielding small yellow crystals, m.p. 101·5–102·5° (Found: N, 11·5. $C_{11}H_{12}N_2O_4$ requires N, 11·9%).

1-(4-*Nitrophenyl*)-2-*nitrohept*-1-*ene* (Compound no. 14)

This was prepared in the same manner as Compound no. 13, but with 1-nitrohexane (6·6 g.). Small straw-coloured crystals were obtained from methanol and then from carbon tetrachloride, m.p. 67–68° (Found: N, 10·2. $C_{13}H_{16}N_2O_4$ requires N, 10·6%).

1-(4-*Nitrophenyl*)-2-*nitronon*-1-*ene* (Compound no. 15)

This was prepared in the same manner as Compound no. 13, but with 1-nitro-octane (8 g.). Straw-coloured plates were obtained from carbon tetrachloride and then from methanol, m.p. 61·5–62·5° (Found: N, 9·8. $C_{15}H_{20}N_2O_4$ requires N, 9·6%).

1-(4-*Nitrophenyl*)-2-*nitrotridec*-1-*ene* (Compound no. 16)

This was prepared in the same manner as Compound no. 13, but with 1-nitrododecane (12·5 g.). Waxy pale yellow plates were obtained from methanol, m.p. 64–65° (Found: N, 7·7. $C_{19}H_{28}N._2O_4$ requires N, 8·0%).

1-(4-*Dimethylaminophenyl*)-2-*nitroprop*-1-*ene* (Compound no. 18)

p-Dimethylaminobenzaldehyde (7·5 g.), nitroethane (3·8 g.) and n-butylamine (0·4 g.) were kept for 2 days. The solid that crystallised out was removed by filtration and recrystallised from benzene, carbon tetrachloride and then ethyl acetate. Glistening brick-red plates were obtained, m.p. 123° (Found: N, 13·8. $C_{11}H_{14}N_2O_2$ requires N, 13·6%).

1-(4-*Dimethylaminophenyl*)-2-*nitrobut*-1-*ene* (Compound no. 19)

This was prepared in the same manner as Compound no. 18, but with 1-nitropropane (4·2 g.). After 11 days the solid that crystallised out was removed by filtration and recrystallised from carbon tetrachloride, ethyl acetate and then methanol. Glistening orange-yellow plates were obtained, m.p. 88–89° (Found: N, 12·4. $C_{12}H_{16}N_2O_2$ requires N, 12·7%).

1-(2-*Furyl*)-2-*nitroprop*-1-*ene* (Compound no. 26)

Freshly distilled furfural (96 g.), nitroethane (75 g.) and 30% w/v solution of methylamine in alcohol (20·3 g.) were kept for 16 h. at 50–55°. The oil was separated from the water of reaction. It solidified on cooling and was recrystallised from alcohol and then carbon tetrachloride to yield yellow crystals, m.p. 51–52° (Found: N, 9·15. $C_7H_7NO_3$ requires N, 9·15%). They resinified to a hard mass on long standing. Attempts were made to nitrate this compound, but a satisfactory product could not be obtained.

1-(2-*Thienyl*)-2-*nitroethylene* (Compound no. 28)

This compound was prepared from 2-thiophenaldehyde[23] (22·4 g.) and nitromethane (12·2 g.) by Worrall's method.[24] Large greenish-yellow rhomboids were obtained from alcohol with the aid of charcoal, carbon tetrachloride and then ethyl acetate, m.p. 80–80·5° (Found: N, 8·7. $C_6H_5NO_2S$ requires N, 9·0%).

An attempt was made to nitrate this compound with fuming nitric acid below −10°. The solid obtained on pouring the nitration mixture on to crushed ice decomposed on attempted purification.

1-(2-*Thienyl*)-2-*nitroprop*-1-*ene* (Compound no. 29)

A mixture of 2-thiophenaldehyde (5·6 g.), nitroethane (3·8 g.) and n-butylamine (0·4 g.) was kept for 11 days. The solid that formed was recrystallised from benzene, carbon tetrachloride and then ethyl acetate. Light brown crystals were obtained, m.p. 67·5° (Found: N, 8·0. $C_7H_7NO_2S$ requires N, 8·3%).

1-(5-*Nitro*-2-*thienyl*)-2-*nitroprop*-1-*ene* (Compound no. 30)

1-(2-Thienyl)-2-nitroprop-1-ene (1·2 g.) was added with stirring for 20 min. to fuming nitric acid (6·5 c.c.) below −8°. The nitration mixture was kept stirred for a further hour below 0° and then poured on to crushed ice. The solid was removed by filtration and dried under reduced pressure. On recrystallisation from acetone light brown crystals were obtained, m.p. 122·5–123° (Found: N, 13·3. $C_7H_6N_2O_4S$ requires N, 13·1%).

1-*Methoxy*-1,3-*di*-(4-*nitrophenyl*)-2,4-*dinitrobutane* (Compound no. 33)

This compound was prepared by the method of Meisenheimer & Heim[25] from 1-(4-nitrophenyl)-2-nitroethylene and sodium methoxide in methanol. After two recrystallisations from methanol short cream-coloured needles were obtained, m.p. 112–113° (Found: N, 1·4.3 $C_{17}H_{15}N_4O_9$ requires N, 13·4%).

1-*Methoxy*-1-(4-*nitrophenyl*)-2-*bromo*-2-*nitropropane* (Compound no. 36)

This compound was prepared by the procedure of Senkus[26] with 1-(4-nitrophenyl)-2-nitroprop-1-ene, bromine, aqueous sodium hydroxide and methanol. On recrystallisation from benzene colourless prisms were obtained, m.p. 170·5–171·5° (Found: N, 9·0. $C_{10}H_{11}BrN_2O_5$ requires N, 8·8%).

Fungitoxicity tests

All the tests were carried out by the Montgomery–Moore[27] slide germination technique, and the values of LD_{95} were determined against *Venturia*, *Botrytis* and *Fusarium*, as described in Part I.[1]

Results

Table I shows the results of fungitoxicity tests on 24 β-nitrostyrenes. The ones substituted in the ring proved more active than the unsubstituted.

From Table II it is evident that also in the heterocyclic (furyl and thiophen) analogues substitution (in the 5-position) by nitro groups enhanced fungitoxicity. Further, the effect of substitution by nitro groups was not limited to ethylenic compounds; it also operated in the saturated compounds, as can be seen from a comparison of activities against *Venturia* of 1-methoxy-1,3-diphenyl-2,4-dinitrobutane (Compound no. 32; **I**, R^I,R^{III} = Ph; R^{II} = OCH_3) with its nitro derivative (Compound no. 33; **I**, R^I,R^{III} = 4-NO_2·C_6H_4, R^{II} = OCH_3) and of 1-methoxy-1-phenyl-2-bromo-2-nitropropane (Compound no. 35; **II**, R^{IV} = Ph) with its nitro derivative (Compound no. 36; **II**, R^{IV} = 4-NO_2·C_6H_4).

$$R^I{\cdot}CHR^{II}{\cdot}CH(NO_2){\cdot}CHR^{III}{\cdot}CH_2{\cdot}NO_2$$

(I)

$$R^{IV}{\cdot}CH(OCH_3){\cdot}C(CH_3)Br{\cdot}NO_2$$

(II)

Table III shows that, in the β-nitrostyrenes, a different structure–activity relationship holds against certain parasitic and saprophytic fungi.

The effect of lengthening the alkyl chain R^v in the system 4-NO_2·C_6H_4·CH:CR^v·NO_2 on the toxicity to *Venturia* is shown in Fig. 1.

Discussion

Bousquet, Kirby & Searle[4] noted the fungitoxicity of nitroethylenes to the saprophytic

Table III

Comparison of toxicity of β-nitrostyrenes to certain parasitic and saprophytic fungi

No. of compound	Name on compound	*Venturia*	*Botrytis*	*Fusarium*	*Penicillium* lumber moulds	*Aspergillus niger*
		LD_{95} values, p.p.m.			LD_{100} values, p.p.m.*	
1	1-Phenyl-2-nitroethylene	320	650	770	17	17
2	1-Phenyl-2-nitroprop-1-ene	490	600	>1000	31	31
3	1-Phenyl-2-nitrobut-1-ene	560	>1000	>1000	62	62
7	1-(4-Chlorophenyl)-2-nitroethylene	96	94	77	31	62
8	1-(4-Chlorophenyl)-2-nitroprop-1-ene	80	150	80	250	250
4	1-(4-Methoxyphenyl)-2-nitroethylene	65	70	70	62	62
20	1-(3-Nitrophenyl)-2-nitroethylene	85	74	100	150	125
25	1-(2-Furyl)-2-nitroethylene	>1000	>1000	>1000	17	17

* Results obtained by Bousquet *et al.*[4]

fungi *Penicillium* lumber moulds and *Aspergillus niger*. Table III shows that the ring-substituted nitrostyrenes were less toxic to these organisms than the unsubstituted ones: 1-phenyl-2-nitroethylene (Compound no. 1) was much more active than its 3-nitro- (Compound no. 20) or its 4-methoxy-derivative (Compound no. 4). Against the parasitic fungi, *Venturia*, *Botrytis* and *Fusarium*, the ring-substituted compounds were more active. Also 1-(2-furyl)-2-nitroethylene (Compound no. 25), found to be highly active against Aspergillus and Penicillium,[4, 6] proved inactive to *Venturia*, *Botrytis* and *Fusarium*. McGowan *et al.*,[6] with a technique (agar) different from the one used here, found 1-phenyl-2-nitroethylene (Compound no. 1) and 1-(2-furyl)-2-nitroethylene (Compound no. 25) to be active also against the parasitic *Botrytis allii* and *Fusarium graminearum*.

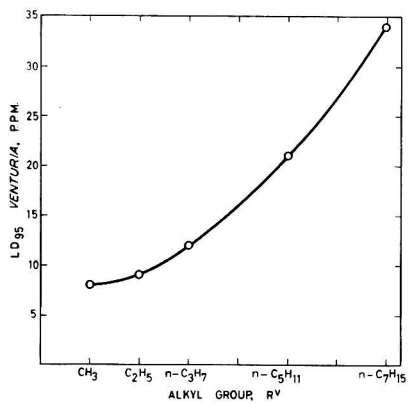

FIG. 1.—*Effect on toxicity to* Venturia, *of lengthening the alkyl chain* R *in the system* $4\text{-}NO_2\cdot C_6H_4\cdot {}^{V}CH{:}CR\cdot NO_2$

The strongly electron-attracting nitro group in the side-chain was found necessary for fungitoxicity. 1-(4-Methoxyphenyl)-2-chloroethylene (Compound no. 31) proved inactive, whereas the 2-nitro-derivative (Compound no. 4) had considerable activity. However, the activity was not limited to the unsaturated compounds. Compounds nos. 35 and 36 (formula **II**), in which the side-chain is fully saturated, possessed good activity against *Venturia*, possibly owing to the reactivity of the bromine towards nucleophilic reagents such as those containing the thiol group.[35, 36]

As already noted, compounds in the nitroethylene series with no substitutents on the rings (nos. 1–3, 25, 26, 28, 29) had poor activity against *Venturia*, *Botrytis* and *Fusarium*. Substitution in the ring enhanced activity. In general, activity increased with increase in

the electronegativity of the substituent and was greatest when the substituent was a nitro group. The effect of substitution by nitro groups was noticeable also in saturated compounds nos. 32 and 33 (formula **I**).

Substitution by an alkyl group on the 2-carbon atom (to which the nitro group is attached) in β-nitrostyrenes, other than those substituted in the ring by a nitro group, had the general effect of lowering the fungitoxicity of the resulting compounds. This may be due to the electron-repelling properties of the alkyl groups counteracting the effect of the 2-nitro group, thus rendering the ethylene bond less reactive to nucleophilic reagents.

That the effect of the alkyl groups is less likely to be of a steric nature is supported by evidence of increased antifungal activity brought about by 2-alkyl substitution in the 1-(4-nitrophenyl)-2-nitroalkene series (Compounds nos. 10–13). The two nitro groups appear to reduce the electronic density on the ethylenic bond to such an extent that it is not materially affected by the electron-donating properties of the alkyl groups, which, as was also found in the carbocyanines[1] and anilinovinyl quaternaries,[2] in fact enhanced the activity (Compounds nos. 10, 11). Had the effect of the 2-alkyl groups been a steric one, the fungitoxicity of this series would have been lowered by substitution with an alkyl group, just as it is in the series in which the ring-substituent is other than a nitro group. The steric effect seems to become operative with the larger alkyl groups (C_7–C_{11}), as shown in Fig. 1.

The introduction of a further electronegative group (—COOEt) (Compound no. 24) does not increase the activity above that of the parent compound.

If in 4,2′-dinitrobiphenyl the 1′,6′-C–C link were broken, a system similar to the one discussed above would obtain. However, 4,2′-dinitrobiphenyl (Compound no. 39) and related compounds proved inactive. For activity it is thus essential that an open chain be available.

Conclusions

On the relationship between fungitoxicity and chemical structure of β-nitrostyrenes and related compounds the following tentative conclusions may perhaps be drawn:

(*a*) substitution in the ring causes an increase in toxicity to the parasitic fungi *Venturia*, *Botrytis*, *Fusarium*;

(*b*) increase in activity is paralleled by an increase in the electronegativity of the ring substituent;

(*c*) substitution by an alkyl group on the 2-carbon atom has the effect of lowering the activity, except when the ring substituent is a nitro group;

(*d*) lengthening of the 2-alkyl substituent in 1-(4-nitrophenyl)-2-nitroalkenes reduces the activity;

(*e*) activity against *Venturia* is retained in compounds in which the ethylenic carbon atoms are saturated with bromine and a methoxy group, respectively;

(*f*) if the nitroethylenic chain is part of a conjugated cycle, activity is lost.

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STUDIES IN FUNGITOXICITY. IV.*—Fungitoxicity of Certain Ethylenic Compounds

By J. D. EDWARDS and M. PIANKA

Several benzylidene and related compounds have been tested for fungitoxicity to certain economically important fungi. They were found to be generally less active than β-nitrostyrenes. Certain electron-attracting groups attached to the ethylenic bond conferred activity, but cyano groups did not.

Introduction

In Part III[1] were described the results of tests of a number of β-nitrostyrenes p-R·C_6H_4·CH:CR′·NO_2 and related compounds for toxicity against the spores of *Venturia inaequalis* (Cooke) Wint. ; *Botrytis cinerea* Pers. ; and *Fusarium bulbigenum*, Cooke & Massee, var. *lycopersici* (Brushi) Wollenw. It was considered of interest to prepare and test compounds in which both groups R′ and NO_2 were replaced by electronegative groups, such as COOEt, COR″, CN, and in which the ring substituent was a chlorine atom or a nitro group in different positions.

Roblin & Hechenbleikner[2] observed the insecticidal activity of benzylidene and furfurylidene malonates and McGowan and co-workers[3] noted the fungistatic activity of ethylenic compounds in which one group—COOR, COR, COOH or CHO—was attached to the ethylenic bond.

* Part III : preceding paper

Experimental

All but the four compounds (nos. 2, 8, 17 and 24) are known and were prepared by methods described in the literature.

Diethyl 4-chlorobenzylidenemalonate (Compound no. 2)

Diethyl malonate (8 g.), *p*-chlorobenzaldehyde (7·02 g.) and piperidine (0·2 g.) were heated on a steam-bath for 6 h. The mixture was then kept at room temperature for 3 days. The water formed was removed from the reaction mixture by distillation with chloroform, and the residue was distilled, to yield a colourless oil (7·7 g.), b.p. 122–124°/0·01 mm. (Found : Cl, 12·2. $C_{14}H_{15}ClO_4$ requires Cl, 12·6%).

4-Chlorobenzylideneacetylacetone (Compound no. 8)

A mixture of acetylacetone (3·55 g.), *p*-chlorobenzaldehyde (5·0 g.) and piperidine (0·02 c.c.) was kept at room temperature for 24 h. 3N-Sulphuric acid (10 c.c.) was added and the mixture extracted with ether. The ether extract was washed twice with water and dried (sodium sulphate) and the ether evaporated. The oily residue solidified after several days and was recrystallised from petroleum, b.p. 60–80° (charcoal). White crystals were obtained, m.p. 73–75·5° (Found : Cl, 15·5. $C_{12}H_{11}ClO_2$ requires Cl, 15·9%).

4-Chlorobenzylidene-dehydracetic acid (Compound no. 17)

p-Chlorobenzaldehyde (5 g.), dehydracetic acid (6 g.), piperidine (0·7 c.c.) and chloroform (50 c.c.) were heated under reflux for 6 h. The flask was then equipped with a Dean & Stark trap to remove the water formed in the reaction and the mixture refluxed for a further 7½ h. The solvent was distilled off *in vacuo*, leaving a solid that on recrystallisation from methanol and then from ethanol yielded yellow needles, m.p. 162–163° (Found : Cl, 12·5. $C_{15}H_{11}ClO_4$ requires Cl, 12·2%).

Diethyl 3,7-dimethyl-5-(3-nitrophenyl)-4,6-dioxanona-2,7-dien-1,9-dioate (Compound no. 24)

m-Nitrobenzaldehyde (4·6 g.), ethyl acetoacetate (3·9 g.) piperidine (3 drops) and ethanol (0·5 c.c.) were kept at −5° to −9° for 5 days, then at room temperature for another 5 days. The crystalline solid that separated was filtered off and recrystallised twice from ethanol. White crystals (3·1 g.) were obtained, m.p. 152–154° (Found : N, 3·3. $C_{19}H_{23}NO_8$ requires N, 3·5%).

Fungitoxicity tests

All the tests were carried out by the Montgomery–Moore[4] slide germination technique, and the values of LD_{95} were determined against *Venturia*, *Botrytis*, *Fusarium* and *Cercospora melonis*, Cooke, as described in Part I.[5]

Results and discussion

Table I shows the results of fungitoxicity tests on 24 ethylenic compounds. The weaker electron-attracting properties of the ethoxycarbonyl and methylcarbonyl groups may be responsible for the activity of compounds nos. 1–9 being lower than that of the β-nitrostyrenes.[1] The specificity of position (*meta*) required for the activity of diethyl nitrobenzylidenemalonate is surprising (Compounds nos. 3 and 4). Other ethylenic compounds derived from *m*-nitrobenzaldehyde (nos. 6, 7, 9, 21, 22) have, however, little or no activity.

Though cyano groups generally contribute to activity against *Venturia*, condensation products of ring-substituted benzaldehydes and ethyl cyanoacetate (Compounds nos. 10–12) or malononitrile (Compounds nos. 13–15) were unexpectedly inactive.

Condensation products of benzaldehyde and of ring-substituted benzaldehydes with dehydracetic acid (nos. 16, 17), with 1-phenyl-3-methyl-5-pyrazolone (no. 22), with fluorene (nos. 18, 19) or with 2,7-dibromofluorene (nos. 20–21) proved to be inactive. Compounds of the acetal type, in which the ethylenic bond was not in conjugation with the aromatic ring (nos. 23, 24), were also inactive.

Table I

Results of fungitoxicity tests with benzylidene compounds

No. of compound	Name of compound	M.p.	Reference	*Venturia*	*Botrytis*	*Fusarium*	*Cerospora*
				LD_{95} values, p.p.m.			
	Diethyl malonate						
1	Benzylidene-	b.p. 138°/0·6 mm.	6	120	>100	200	>100
2	4-Chlorobenzylidene-	b.p. 122–124°/0·01 mm.		80	>1000	>100	>100
3	4-Nitrobenzylidene-	93°	7	>1000	>1000	>1000	>1000
4	3-Nitrobenzylidene-	75°	8	25	>1000	80	>1000
	Ethyl acetoacetate						
5	4-Chlorobenzylidene-	85–87°	18	130	540	180	100
6	3-Nitrobenzylidene-	110°	9	200	>1000	80	>100
	Ethyl benzoylacetate						
7	3-Nitrobenzylidene-	106–107°	8	100	>1000	>1000	>1000
	Acetylacetone						
8	4-Chlorobenzylidene-	73–75°		130	540	170	100
9	3-Nitrobenzylidene-	101–102°	10	180	>1000	440	180
	Ethyl cyanoacetate						
10	4-Chlorobenzylidene-	93°	11	>1000	>100c	>1000	>1000
11	4-Nitrobenzylidene-	169–170°	12	>1000	>1000	>1000	>1000
12	3-Nitrobenzylidene-	130–133°	13	>1000	>1000	>1000	>1000
	Malononitrile						
13	4-Chlorobenzylidene-	162–163°	14	400	>1000	200	1000
14	4-Nitrobenzylidene-	161–162°	15	>100	>1000	1000	>100
15	3-Nitrobenzylidene-	103–104°	16	1000	>1000	>1000	>1000
	Dehydracetic acid						
16	Benzylidene-	130–131°	17	>1000	>1000	>1000	>1000
17	4-Chlorobenzylidene	162–163°		>1000	>1000	>1000	>1000
	Fluorene						
18	4-Chlorobenzylidene-	148–149°	19	>1000	>1000	>1000	>1000
19	3-Nitrobenzylidene-	111–112°	20	>1000	>1000	>1000	>1000
	2,7-Dibromofluorene						
20	4-Chlorobenzylidene-	215–216°	19	>1000	>1000	>1000	>1000
21	3-Nitrobenzylidene-	155–156°	19	>1000	>1000	>1000	>1000
	1-Phenyl-3-methyl-5-pyrazolone						
22	3-Nitrobenzylidene-	157–158°	21	>1000	>1000	>1000	>1000
	Miscellaneous compounds						
23	Diethyl 3,7-dimethyl-5-(4-chlorophenyl)-4,6-dioxanona-2,7-dien-1,9-dioate	156°	18	>1000	>1000	>1000	>1000
24	Diethyl 3,7-dimethyl-5-(3-nitrophenyl)-4,6-dioxanona-2,7-dien-1,9-dioate	152–154°		>1000	>1000	>1000	>1000

Conclusions

From the very limited results available the following very tentative conclusions may perhaps be drawn on the relationship between fungitoxicity to the parasitic fungi: *Venturia, Botrytis, Fusarium* and *Cercospora,* and chemical structure of certain benzylidene compounds:

(*a*) ethylenic compounds in which ethoxycarbonyl and methylcarbonyl groups are attached to the ethylenic bond are less active than those with a nitro group ;[1, 3]

(*b*) ethylenic compounds in which the electronegative CN is attached to the ethylenic bond are inactive ;

(*c*) benzylidene compounds in which the double bond carries a carbonyl heterocyclic group or a fluorene group are inactive.

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CITRUS ESSENTIAL OILS. III.*—Evaluation of Sicilian Natural Lemon Oils

By C. A. SLATER

An examination of the infra-red spectra of the oxygenated fractions of Sicilian natural lemon oils has made possible a new method of evaluation which agrees very closely with the results obtained by classical methods of analysis and organoleptic testing. In addition, methods are suggested for the detection of adulteration of natural lemon oils.

Introduction

The first paper in this series[1] described a method for the evaluation of natural and terpeneless lemon oils by physical methods. Since then many more samples of Sicilian natural lemon oil have been examined, and certain weaknesses in the previous method have been found. Re-appraisal of the results has given better criteria and a physical method is now described for the evaluation of natural lemon oils, which shows a very high degree of agreement with results obtained by classical methods.

* Part II: *J. Sci. Fd Agric.*, 1961, **12,** 732

Experimental

Gas chromatography.—Analytical and preparative gas chromatography were carried out as previously described.[2]

Infra-red spectroscopy.—The oxygenated fractions of the oils were isolated as previously described,[1] and the infra-red spectra recorded for thin films between rock salt plates, using a Hilger & Watts H.800 Spectrophotometer. The ratios of the peak heights (measured as shown in Fig. 1) at 2·95 and 5·95 μ (peaks 1 and 2 ; deterioration index[1]) ; 6·12 and 6·20 μ (peaks 3 and 4) ; and 8·66 and 8·91 μ (peaks 5 and 6) were calculated.

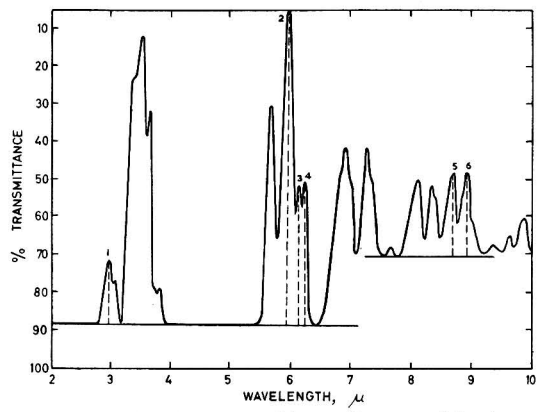

FIG. 1.—*Infra-red spectrum of lemon oil oxygenated fraction*
Ratios of peak heights—1 : 2, deterioration index
3 : 4, 6·12–6·20 μ
5 : 6, 8·66–8·91 μ

Isolation of citral.—Samples of Sicilian natural lemon oil and of lemongrass oil were separated into their oxygenated and hydrocarbon fractions as previously described.[1] Citral was isolated from the oxygenated fractions by preparative gas chromatography. Analysis of these two citral fractions by gas chromatography showed that from Sicilian lemon oil to contain 65·9% citral a and 34·1% citral b, and that from lemongrass oil to contain 64·8% citral a and 35·2% citral b. The infra-red spectra of the two citrals were identical with that of the commercial citral (Fig. 2*c*).

Isolation of citrals a and b.—Commercial citral (0·5 ml.) was chromatographed on the preparative gas column and samples of citrals a and b, approximately 97% pure as judged by analytical gas chromatography, were isolated. Their infra-red spectra, together with that of the original mixture, are given in Fig. 2.

Results and discussion

(1) *Evaluation*

Since the publication of Part I[1] of this series, the infra-red spectra of the oxygenated fractions of a further 64 Sicilian natural lemon oils have been examined. A better evaluation of those oils previously found by classical methods of analysis to be sometimes acceptable and sometimes not acceptable is now possible. The new method of evaluation is based on the percentage of oxygenated compounds in each oil and the ratios of heights of three pairs of absorption bands in the infra-red (Fig. 1) indicated above.

A comparison of a number of oils gave the following limits for the acceptability of an oil for use as a flavouring material.

Oxygenated compounds, %	8–10%
Deterioration index	<0·30
Peak height at 6·12/peak height at 6·20 μ	0·94–0·98
,, ,, ,, 8·66/ ,, ,, at 8·91 μ	0·97–1·05

An oil with figures within the stated limits is of good quality and is regarded as acceptable for use in lemon flavours in the Soft Drinks Industry. Small deviations from these limits in the present work have resulted in the oil being labelled as ' borderline '.

Table I lists the results for 42 genuine Sicilian natural lemon oils. It can be seen that in all cases where an oil is rejected by classical methods of analysis it is also rejected by the physical method described above. Twenty-one samples are found to be acceptable by classical analysis and of these fifteen are acceptable on the basis of physical measurements, three are borderline, and three are rejected.

Eleven oils are placed in a borderline category by classical methods and of these six were also borderline on the basis of physical measurements whilst three are acceptable and two rejected.

The overall agreement in the results is very good, and there is no doubt that oils selected by the physical method alone could quite safely be used for the production of good lemon flavours for use in soft drinks.

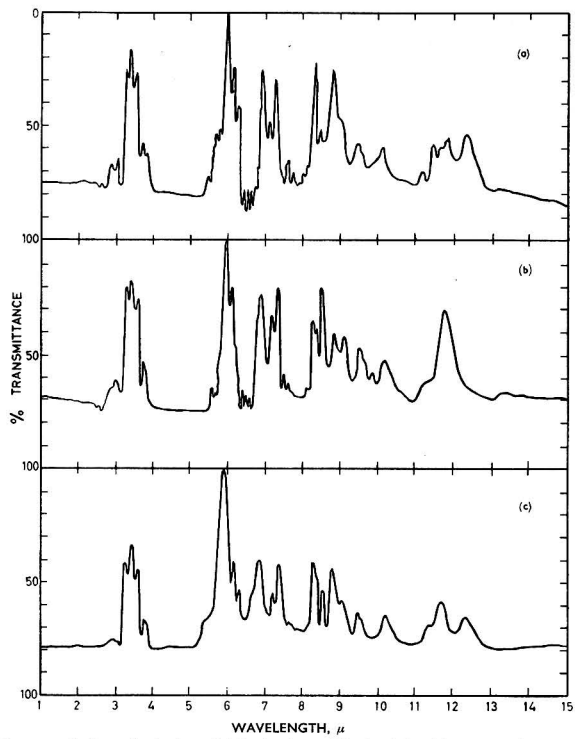

FIG. 2.—*Infra-red spectra of* (a) *citral a;* (b) *citral b;* (c) *commercial citral*

(2) *Adulteration*

(*a*) *Citral.*—The infra-red spectra of commercial citral samples isolated from lemon oil or from lemongrass oil are identical (Fig. 2*c*), and show the presence of strong bands at 8·38 and 8·91 μ of roughly equal intensity, and of a weaker band at 8·66 μ. The bands at 8·38 and 8·91 μ are attributable to citral a (Fig. 2*a*) and that at 8·66 μ to citral b (Fig. 2*b*).

The ratio of the peaks at 8·66 and 8·91 μ in lemon oil, while nearly constant, is very much higher than in natural citral indicating that in lemon oil the peak at 8·66 μ is not due to citral alone but probably to the presence of an unknown ester in addition. Similarly, the ratio of the peaks at 6·12 and 6·20 μ in lemon oil is almost constant but is lower than would be expected if these peaks were due to citral only.

Table I

Analysis of genuine Sicilian natural lemon oils

Sample no.	Oxygenated compounds, %	Deterioration index	Ratio of peak heights		Whether acceptable	
			6·12/6·20 μ	8·66/8·91 μ	(a) Classical method	(b) Physical method (this paper)
1	9·4	0·14	0·94	1·03	Yes	Yes
2	9·1	0·19	0·95	1·00	Yes	Yes
3	8·8	0·17	0·96	1·00	Yes	Yes
4	8·0	0·21	0·94	1·00	Yes	Yes
5	8·0	0·22	0·95	1·03	Yes	Yes
6	8·1	0·14	0·98	1·05	Yes	Yes
7	9·4	0·25	0·95	1·00	Yes	Yes
8	10·0	0·30	0·96	0·97	Yes	Yes
9	7·9	0·30	0·96	1·05	Yes	Yes
10	7·9	0·24	0·94	1·00	Yes	Yes
11	8·2	0·20	0·95	1·00	Yes	Yes
12	8·8	0·16	0·95	1·00	Yes	Yes
13	8·7	0·10	0·95	1·02	Yes	Yes
14	9·0	0·15	0·97	1·01	Yes	Yes
15	9·9	0·18	0·98	0·98	Yes	Yes
16	10·7	0·30	0·96	1·00	Yes	Borderline
17	10·2	0·31	0·98	1·00	Yes	Borderline
18	8·1	0·26	0·96	1·08	Yes	Borderline
19	12·9	0·16	1·00	2·10	Yes	No
20	10·4	0·40	0·98	1·00	Yes	No
21	9·1	0·18	0·93	0·92	Yes	No
22	9·1	0·19	0·95	1·00	Borderline	Yes
23	8·8	0·16	0·95	1·02	Borderline	Yes
24	8·9	0·12	0·94	1·02	Borderline	Yes
25	9·1	0·36	1·05	1·04	Borderline	No
26	8·2	0·14	0·92	1·30	Borderline	No
27	10·8	0·18	0·98	1·00	Borderline	Borderline
28	7·7	0·24	0·94	1·00	Borderline	Borderline
29	10·0	0·19	0·98	1·06	Borderline	Borderline
30	7·8	0·13	0·94	1·00	Borderline	Borderline
31	8·3	0·17	0·92	1·02	Borderline	Borderline
32	8·2	0·17	0·92	1·06	Borderline	Borderline
33	13·8	0·30	0·90	0·92	No	No
34	14·1	0·45	0·96	0·91	No	No
35	9·7	0·24	1·00	1·20	No	No
36	3·5	0·28	0·88	1·20	No	No
37	4·3	0·25	0·88	1·20	No	No
38	7·5	0·16	1·00	0·94	No	No
39	8·1	0·19	1·00	0·96	No	No
40	9·4	0·18	1·00	1·04	No	No
41	7·3	0·15	0·91	1·00	No	No
42	7·9	0·25	0·97	1·43	No	No

It follows, therefore, that addition of commercial citral to a lemon oil should result in a drop in the ratio of peak heights at 8·66 and 8·91 μ and a rise in the ratio of heights at 6·12 and 6·20 μ. Samples 48–52 (Table II and Fig. 4*c*) show the effect of adding citral to a Sicilian lemon oil with a somewhat low citral value (3·22%) (Sample 31, Tables I and II, a borderline oil). It is clear that, as expected, addition of citral resulted in a reduction in the ratio of peak heights at 8·66 and 8·91 μ and a rise in the ratio of heights at 6·12 and 6·20 μ.

When the figures obtained in these experiments are compared with those for three commercial oils known to contain added citral (Table II, samples 43–45), it is seen that similar

ratios are found. It seems reasonable to conclude that such changes will occur in any oil adulterated with citral, and hence it is highly likely that Samples 46 and 47 (Table II) are also adulterated in this way.

Table II

Analysis of Sicilian natural lemon oil adulterated with citral

Sample no.	Oxygenated compounds, %	Ratio of peak heights 6·12/6·20 μ	Ratio of peak heights 8·66/8·91 μ	Whether acceptable by Classical method	Whether acceptable by Physical method	Whether adulterated
43[a]	11·4	1·40	0·87	No	No	Yes
44[a]	12·8	1·30	0·83	No	No	Yes
45[a]	12·3	1·30	0·88	No	No	Yes
46[a]	10·3	1·25	0·80	No	No	Uncertain
47[a]	8·9	1·00	0·84	Borderline	No	Uncertain
31	8·3	0·92	1·02	Borderline	Borderline	No
48[b]	10·3	1·00	0·94	Not tested	No	Yes (0·78%)
49[b]	11·3	1·06	0·94	Not tested	No	Yes (1·49%)
50[b]	9·4	1·06	0·88	Not tested	No	Yes (1·99%)
51[b]	12·2	1·09	0·88	Not tested	No	Yes (2·49%)
52[b]	11·6	1·14	0·85	Not tested	No	Yes (3·31%)
Commercial citral	—	1·65	0·66	—	—	—

[a] Commercial samples of Sicilian lemon oil
[b] Borderline oil no. 31 (3·22% citral) to which additional citral was added—amount added in parentheses

(*b*) *Benzyl alcohol.*—In Table III are shown the results of the analysis of twelve cheap unacceptable Sicilian lemon oils. These oils are all remarkable in that the oxygenated fractions have strong infra-red absorptions in the 13–15 μ region (Fig. 4*d*) indicative of the presence of a benzenoid adulterant (cf. Fig. 4*a*, the infra-red spectrum of the oxygenated fraction from a genuine, good Sicilian lemon oil). In addition, gas chromatography reveals in these oils an unusual balance of citral a to citral b (Fig. 3*b*) as compared with a good natural lemon oil (Fig. 3*a*) (cf. [4]). The ' citral b ' fraction (B, Fig. 3*b*) (0·3 ml.) from one of these cheap oils was isolated by preparative gas chromatography and separated by adsorption chromatography on silica gel (Hopkins & Williams M.F.C. 37 × 1 cm.). By elution with successive portions (50 ml.) of light petroleum (b.p. 30–40°)/ether mixtures, three fractions were obtained. These were identified by infra-red spectroscopy as citral b (light petroleum ether/ether 90 : 10), α-terpineol (light petroleum/ether 80 : 20), and (major component) benzyl alcohol (ether).

Table III

Analysis of Sicilian natural lemon oils having strong infra-red absorptions in the 13–15 μ region

Sample no.	Oxygenated compounds, %	Deterioration index	Ratio peak heights 6·12/6·20 μ	Ratio peak heights 8·66/8·91 μ	Whether acceptable classical method	Whether acceptable physical method
53	9·5	0·43	1·00	0·97	No	No
54	9·4	0·35	0·98	0·96	No	No
55	14·4	0·34	0·98	1·07	No	No
56	11·6	0·37	1·08	0·94	No	No
57	14·2	0·41	0·88	1·40	No	No
58	11·4	0·43	1·60	1·00	No	No
59	9·4	0·63	1·05	1·04	No	No
60	11·2	0·39	1·07	1·04	No	No
61	8·7	0·31	1·25	0·92	No	No
62	9·5	0·22	1·01	0·98	No	No
63	8·2	0·27	0·94	1·10	No	No
64	10·0	0·31	1·07	1·02	No	No

No trace of benzyl alcohol has been found in known genuine Sicilian lemon oils and this material must be regarded as an adulterant.

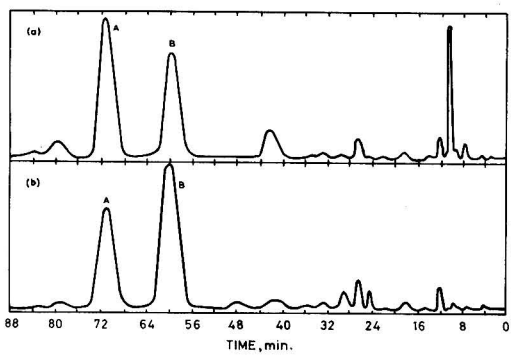

FIG. 3.—*Gas chromatograms of oxygenated fractions of Sicilian lemon oils,* (a) *genuine oil;* (b) *oil adulterated with benzyl alcohol*

A, citral a; B, citral b

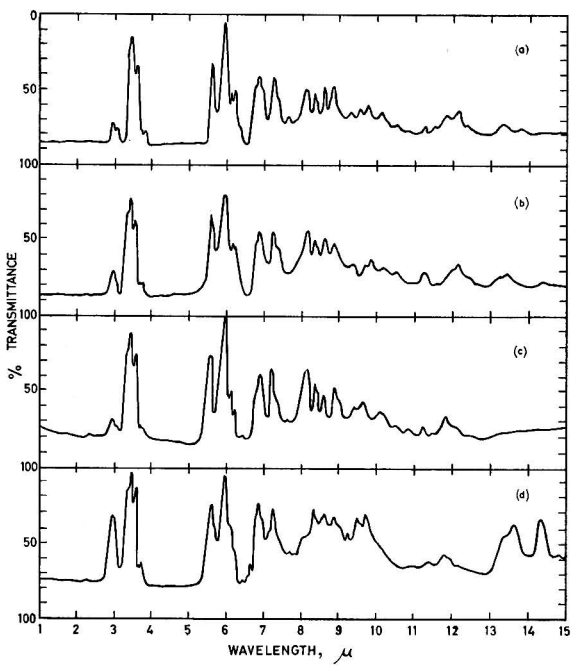

FIG. 4.—*Infra-red spectra of Sicilian lemon oil oxygenated fractions,* (a) *acceptable oil;* (b) *unacceptable oil;* (c) *oil adulterated with citral;* (d) *oil adulterated with benzyl alcohol*

It is clear that adulteration of this type may be detected readily by a rapid examination of the infra-red spectrum of the oxygenated fraction of the oil and can be confirmed by examining the gas chromatography pattern of the same fraction.

Conclusion

In Fig. 4 the infra-red spectra of the oxygenated fractions of natural Sicilian lemon oils which are acceptable (Fig. 4*a*), genuine but unacceptable (Fig. 4*b*), adulterated with citral (Fig. 4*c*), and adulterated with benzyl alcohol (Fig. 4*d*) are compared with one another. These spectra together with the results reported in the Tables show that it is possible to evaluate natural lemon oils by physical methods. In addition, adulteration of the oils by the addition of citral or benzyl alcohol may be readily detected.

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ERRATUM

In the paper by Storry & Rook (*J. Sci. Fd Agric.*, 1962, **13**, 621) the first lines of the Discussion on p. 626 should read ' In spite . . . body of the dairy cow, only a small proportion—probably not more than 2 g. in the skeleton and 3 g. in the soft tissues—can be drawn upon . . .'

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JANUARY, 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

JANUARY, 1963

I.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Two-dimensional analysis of the 'stand-pipe' method of determining the permeability of a soil. C. M. Segedin and J. B. Miller (*N.Z. J. Sci.*, 1962, **5**, 43—53).—The flow from the bottom of a lined ditch into a uniform permeable soil is analysed and may be considered as a two-dimensional model of the 'stand-pipe' method of measuring the permeability coeff. of a soil *in situ*.
E. G. BRICKELL.

Woburn irrigation, 1951—9. I. Purpose, design and weather. II. Results for grass. III. Results for rotation crops. H. L. Penman (*J. agric. Sci.*, 1962, **58**, 343—348, 349—364, 365—379).—I. The outline of the design of the experiment is given and the theoretical basis restated. Over a period of 9 years, irrigation was required in five seasons, 18 out of the 20 crops in these years responding to irrigation. Four of the 16 crops grown in the other years responded to irrigation—a few others negatively.

II. Water increased clover production; N decreased it. Both treatments increased total dry matter production in a mixed grass–clover ley. When water supply was non-limiting, the growth rate was proportional to the potential transpiration rate, the accumulated values of each when plotted gave a straight line with slope representing the response of the crop to 1 in. irrigation when this was needed. When water supply was limiting, doubling the N dressing acted as an extra $\frac{1}{2}$ in. of readily available soil water. If periods of leaching are excluded, the mean response in wt./acre/in. was a little smaller than the theoretical limit, obtained from the non-limiting water plots. In a given year the N content of the crop was independent of irrigation and up to 80 cwt./acre/annum of growth was insensitive to K.

III. Yield of potatoes plotted against adjusted potential transpiration, gives straight lines fitting both early and late crops. The limiting deficit was 1 in., drier soils giving growth checks. The average loss per day delay in planting, using a mean date of 22nd April was 3 cwt./acre. The estimated efficiency of photosynthesis from time of full cover was of the order of 10^{-2}. Early potatoes responded to irrigation every year, no difference existing between early and late watering. Main crops, however, gave a variable response. The limiting deficit for sugar beet appeared to be about 2 in. at singling, increasing to about 3 in. by the end of Sept. Top growth was governed by weather from emergence onwards, whilst root growth and sugar yield was governed by weather only from singling onwards. Response to irrigation was variable. Extra N was more effective than irrigation in increasing barley yields, but wheat required more water before it could respond to extra N. The limiting deficit for spring beans varied from 1 to 2·3 in. Irrigation produced a tall crop and delayed ripening. Farmyard manure behaved as an addition of 0·7 in. of available water, but gave a positive effect only if water was limiting.
M. LONG.

Action of salts on disintegration of soils. W. Hutter (*C. R. Acad. Agric. Fr.*, 1962, **48**, 186—189).—Laboratory experiments are described in which a soil was crushed, sieved and compressed into cylinders before or after wetting with water or solutions of NaCl, $MgCl_2$ or $CaCl_2$. In both series the disintegrating effect of NaCl on the structure and cohesion of drying soil was much greater than the effect of $CaCl_2$ or $MgCl_2$. The cylinders moistened and dried after compression were more resistant to pressure than were those dried during compression.
P. S. ARUP.

Effect of leaching saline alkali soils with irrigation waters of different kinds on the permeability and composition of the soils and the composition of the leachates. P. B. L. Srivastava, C. L. Mehrotra and R. R. Agarwal (*J. Indian Soc. Soil Sci.*, 1962, **10**, 93—98).—Various soils were leached with water from different sources with and without additions of gypsum. The permeability of soils was greatest when gypsum was added and least when well-water practically free from salts was used: the soil pH was increased by well-water and lowered by gypsum. Sewage effluent was notably effective in lowering soil salinity.
A. G. POLLARD.

Sodium carbonate and absorbed sodium in semi-arid soils. W. P. Kelley (*Soil Sci.*, 1962, **94**, 1—5).—The fundamental chemistry of the occurrence and reactions of Na_2CO_3 in soil are discussed in relation to base-exchange phenomena.
A. G. POLLARD.

Diffusion of strontium-90 and caesium-137 in soils. T. C. Juang, C. H. Chang and T. M. Lai (*Rep. Taiwan Sug. Exp. Sta.*, 1962, No. 27, 55—66).—Measured in various samples of Taiwan soils the highest diffusion coeff. of ^{90}Sr obtained was $4{\cdot}41 \times 10^{-7}$ cm.^{2} sec.$^{-1}$ and of ^{137}Cs $0{\cdot}27 \times 10^{-7}$ cm.^{2} sec.$^{-1}$. In some soils the diffusion of ^{137}Cs was too low to measure. Diffusion coeff. increased with increasing moisture content and in the case of ^{90}Sr were greatly influenced by exchangeable cations. In reference clays the diffusion coeff. of ^{137}Cs and ^{90}Sr were $6{\cdot}54 \times 10^{-7}$ cm.^{2} sec.$^{-1}$ and $3{\cdot}99 \times 10^{-7}$ cm.^{2} sec.$^{-1}$ respectively in Na-bentonite gel and $0{\cdot}87 \times 10^{-7}$ cm.^{2} sec.$^{-1}$ and $4{\cdot}26 \times 10^{-7}$ cm.^{2} sec.$^{-1}$ respectively in Na-kaolinite. (15 references.)
W. ELSTOW.

Effects of heavy lime applications to volcanic ash soils in the humid tropics. A. J. Rixon and G. D. Sherman (*Soil Sci.*, 1962, **94**, 19—27).—In soils from three different series field plots were limed to varying levels with crushed coral ($CaCO_3$, 95%), several rates of application of superphosphate being superimposed. In general, the pH of the soils increased but in no case was neutrality attained at the end of five months. Increases in exchangeable Ca were generally associated with diminution in exchangeable Al, a significant correlation being established. Negative correlation between exchangeable Al and pH and a positive relation between log exchangeable Ca and pH were established. Liming caused no appreciable change in base exchange capacity. Phosphate treatments produced different effects according to soil type, e.g., increased base-exchange capacity in one group and decreased exchangeable Ca in others. The action of PO_4^{3-} was usually limited to a particular range of application of coral.
A. G. POLLARD.

Lime potential. II. Relation between lime potential and per cent base saturation of negatively charged clays in aqueous salt suspensions. R. C. Turner and W. E. Nichol (*Soil Sci.*, 1962, **94**, 58—63).—Clays saturated with Fe behaved in the same way as those saturated with Al when titrated with $Ca(OH)_2$ but the lime potential (*LP*) thus determined was lower in the Fe clays. In Al-saturated clays the Al is replaceable by other trivalent cations having relatively insol. hydroxides without affecting the relation between *LP* and percent saturation with bases; the magnitude of the *LP* in such circumstances is dependent on the solubility of the hydroxide of the cation concerned. Data obtained with bentonite indicated that the *LP* may not always be a correct measure of base saturation: it indicates whether an appreciable part of the permanent charge on a clay is neutralised by Al or Fe.
A. G. POLLARD.

Research on redox potentials of paddy soils in Japan. Shigenori Aomine (*Soil Sci.*, 1962, **94**, 6—13).—A review and summary of the main current conclusions on the effects of water-logging of soils. (36 references.)
A. G. POLLARD.

Loss of mineral nitrogen from soil. K. Shaw (*J. agric. Sci.*, 1962, **58**, 145—152).—In light sandy soils, without vertical structural fissures, NO_3^- (**I**) is distributed down the profile on leaching, whilst with heavy soils, well fissured and with a gleyed horizon at cultivation depth, **I** largely disappears from the profile on removal from the cultivation layer. Light and heavy soils require the same amount of rain to move **I** from the surface layer; heavy and continuous rain is necessary for complete removal. NH_4^+-N is not leached from either type of soil. Less rain is required to leach **I** in winter than in summer.
M. LONG.

Soil phosphate and the delimitation of plant communities in eastern Australia. II. N. C. W. Beadle (*Ecology*, 1962, **43**, 281—288; cf. *ibid.*, 1954, **35**, 370).—Further data are presented supporting the author's view that the P level of soil is a major factor delimiting the rain-forests, wet sclerophyll forests, xeromorphic low forests, woodlands and scrubs. Fixation of N in the soils is also controlled by the P status.
A. G. POLLARD.

Effect of soil-water stress on the absorption of soil phosphorus by wheat plants. R. G. Fawcett and J. P. Quirk (*Aust. J. agric. Res.*, 1962, **13**, 193—205).—Growth by young wheat plants on a lateritic podsolic soil showed that absorption was not affected by increasing soil-water stress, provided the plants were not damaged by wilting. Available soil P was derived mainly from fine pores undrained at suctions approaching 15 atm. Absorption of P increased with increasing soil P potential for all levels of water stress. (18 references.)
E. G. BRICKELL.

Effect of soil amendment (EDTA) on uptake of soil- and fertiliser-phosphorus by wheat, rice and berseem. N. P. Datta, J. E. Shindl, M. B. Kamath and S. K. DeDatta (*J. Indian Soc. Soil Sci.*, 1962, **10,** 121—128).—Incubation of soil samples with EDTA (Na salt) did not affect the pH but in most cases increased the available PO_4^{3-} (Olsen). In cropping trials application of EDTA (up to 200 lb./acre) increased the dry matter yields and uptake of P by rice and wheat in some but not in all soils; berseem was unaffected by the treatment in any soil. The uptake of fertiliser-P by wheat and berseem was unaffected by EDTA. A. G. POLLARD.

Correlation between soil test values for potassium and crop responses to potassic fertilisers by paddy and wheat in Indian soils. P. K. Oommen (*J. Indian Soc. Soil Sci.*, 1962, **10,** 155—159).—Comparison is made of various extractants (Morgan's, 0·5N-HNO_3, 1% citric acid, neutral N-NH_4OAc) for available K. None of the extractants was effective in all of a range of soils carrying different crops under examination. A. G. POLLARD.

Available potassium in Indian soils as estimated by different extractants. P. K. Oommen and V. Iswaran (*Soil Sci.*, 1962, **94,** 44—47).—Comparative data from 20 soils using five extractants are recorded. Results using Morgan's extractant and 0·5N-HNO_3 (30 min. shaking) were highly correlated, whereas the latter procedure and the neutral N-NH_4OAc method for exchangeable K gave results correlated significantly only at the 5% level. When light soils only were considered, 0·5N-HNO_3 (heating 1 h.) yielded values highly significantly correlated with the exchangeable K. Taking all soils together close correlation (1%) was established between values for 0·5N-HNO_3 (30 min. shaking) and those for the same extractant with 1 h. heating. A. G. POLLARD.

Soil magnesium. I. Laboratory investigation into the displacement and replacement of magnesium in soils. D. E. Hogg (*N.Z. J. Sci.*, 1962, **5,** 64—73).—Displacement was greatest on sandy and pumice soils but very little displacement occurred if K salts having CO_3^{2-}, HCO_3^-, or PO_4^{3-} as the anion, were applied. Mg^{2+} contributed to soils by serpentine superphosphate approximated to the water-sol. content of the superphosphate applied; average total and average water sol. contents of the latter were 5·6 and 2·3% respectively. E. G. BRICKELL.

Chloride content of Rhodesian soils. P. M. Grant, D. H. Saunder and R. M. Gordon (*Rhod. agric. J.*, 1961, **58,** 348—349).—The Cl^- content of Rhodesian soils [100 g. of air-dried soil leached with 250 ml. of 2% $Ca(NO_3)_2$] averaged 4·9 p.p.m. in top- and 3·6 p.p.m. in sub-soils. High Cl^- (up to 33 p.p.m.) occurred in soils with defective drainage (poorly structured sedimentary and vlei soils). A. H. CORNFIELD.

Chromatographic approach to the determination of sulphate absorption and exchange of less-retentive soils. S. C. Fang, R. D. Nance, Tsun T. Chao and M. E. Harward (*Soil Sci.*, 1962, **94,** 14—18).—Interactions between sol. SO_4^{2-} and soils were examined by percolation procedures in soil columns using ^{35}S as a marker for calculating the extent of absorption, exchangeable and fixed SO_4^{2-}. The equilibrium coeff., soil SO_4^{2-}/sol. SO_4^{2-}, tended to be constant for each of the poorly-retentive soils. Adsorption of SO_4^{2-} was in accord with the Freundlich isotherm. Adsorbed $^{35}SO_4^{2-}$ was almost completely exchangeable although the rate of exchange varied with soil type. The amount of non- or difficultly-exchangeable SO_4^{2-} increased with rise in concn. of the SO_4^{2-} solution with which the soil was in equilibrium. Two sites of adsorption of SO_4^{2-} were detectable in 12 soils examined, adsorption at one of the sites being in kinetic equilibrium with that at the other. In the remaining eight soils only one site of adsorption was apparent. A. G. POLLARD.

Availability of copper from copper-humic acid complexes. M. T. Ennis and J. C. Brogan (*Irish J. agric. Res.*, 1961, **1,** 35—42).—The availability of Cu in three complexes is examined by acid (0·01N-HCl) and by pot experiments with oats. The complexes showed marked differences in availability which was much less than that of $CuSO_4$ in all cases and approached nil in one instance. Extraction of the complexes with different concn. of HCl serves to measure the availability of Cu. A. G. POLLARD.

Adjusting soil solutions to specified boron concentrations. J. T. Hatcher, G. Y. Blair and C. A. Bower (*Soil Sci.*, 1962, **94,** 55—57).—A method is described for maintaining specified concn. of B in soil solutions for plant growth investigations, by establishing the relationship between concn. of soil solutions obtained by the pressure membrane technique and the amount of B added to soil. Fixation of B is minimised by avoiding undue drying of the soil. A. G. POLLARD.

Selenium in Irish soils and plants. G. A. Fleming (*Soil Sci.*, 1962, **94,** 28—35).—Areas in which the vegetation is particularly toxic to animals occur mainly on low-lying, poorly-drained soils of high org. matter content. In pot experiments the uptake of Se by clovers, grasses, cereals and vegetables are determined. The possible risk to public health of the consumption of garden vegetables from these soils is noted. A. G. POLLARD.

Determination of streptomycin in soil and the effect of soil colloidal material on its activity. D. Pramer and R. L. Starkey (*Soil Sci.*, 1962, **94,** 48—54).—The method is based on the inhibition by a suspension (**I**) of the soil under test of two strains of *Esch. coli*, one dependent on and the other sensitive to **I**. Actinomyces are added to eliminate Gram-positive sporing organisms without affecting the test organisms. The method permits determination of **I** adsorbed on soil colloids, or on ion-exchange resins but only 40% of that on activated C. Soil pH in the range 4·5—8·5 did not affect the assay. A. G. POLLARD.

Micronutrient deficiencies in the United States. K. C. Berger (*J. agric. Fd Chem.*, 1962, **10,** 178—181).—The frequency of micronutrient deficiencies of B, Cu, Fe, Mn, Mo and Zn in the U.S.A. and the crops and states concerned are listed. B deficiency is most widespread because available B is easily leached from the soil. It is replaced by microbial action on org. matter; intermittent B deficiency often occurs in periods of drought due to lack of microbial action. W. ELSTOW.

Chemistry and availability of micronutrients in soils. F. G. Viets, jun. (*J. agric. Fd Chem.*, 1962, **10,** 174—178).—It is postulated that micronutrients may be present in any one of the following five states (represented as pools) revealed by analysis: (*a*) water sol., (*b*) cations exchangeable by a weak exchanger, e.g. NH_4^+, (*c*) adsorbed, chelated or complexed ions, (*d*) cations in secondary clay minerals together with insol. oxides and (*e*) cations held in primary clay minerals. Thus a soil may contain an ample quantity of an element in pool (*e*), but which is totally unavailable. If plants can absorb ions only from solution the availability of micronutrients is governed by the size of (*a*) and the rate at which elements in other pools may be transferred to (*a*). (28 references.) W. ELSTOW.

Selectivity coefficients of trace elements on a montmorillonite clay and a humic acid system. A. N. Basu, B. K. Seal and S. K. Mukherjee (*J. Indian chem. Soc.*, 1962, **39,** 71—78).—The equilibria between the H^+ forms of the ion-exchangers montmorillonite and humic acid and different concn. of sulphate solutions of Cu^{2+}, Ni^{2+}, Co^{2+}, Cr^{3+}, Zn^{2+} and Mn^{2+} were studied as well as the equilibria when these metals on the ion-exchanger were exchanged against the sulphates of Na^+, K^+, NH_4^+ and Mg^{2+}. The base-exchange capacities and exchangeable base contents of the ion-exchangers were determined together with the selectivity coeff. and the order of elution of the metal cations. The results show a variation of the selectivity coeff. with changes in the equilibrium concn., particularly at low concn. and point to a heterogeneous character of the surfaces of the ion-exchangers. The relative behaviour of the various cations is given by comparison of their selectivity coeff. in equilibrium conditions at the saturation capacities of the ion-exchangers. J. I. M. JONES.

Properties of chelates and their use in crop production. J. C. Brown and L. O. Tiffin (*J. agric. Fd Chem.*, 1962, **10,** 192—195).—A short review of the literature dealing with the problem of making insol. Fe available to plants by the use of various chelating agents. Roots and chelating agents compete for the Fe in a nutrient solution and the roots which compete most effectively appear to reduce Fe^{3+} to Fe^{2+}. (32 references.) W. ELSTOW.

Problems of soil microbiology in increasing the productivity of agriculture. E. N. Mishustin (*Mikrobiologiya*, 1962, **31,** 362—370).—Problems in further development of Soviet agriculture include intensified mechanisation and the study of microbiological processes in soil in relation to plant growth. As examples are quoted the mycorrhiza associated with woodland species and various prep. containing cultures of micro-organisms. P. W. B. HARRISON.

Factors limiting microbial activities in soil. I. Level of substrate, nitrogen and phosphate. II. Effect of sulphur. G. Stotzky and A. G. Norman (*Arch. Mikrobiol.*, 1961, **40,** 341—369, 370—382; cf. *Proc. Soil Sci. Soc. Amer.*, 1958, **22,** 270—271).—I. The decomposition of glucose and of native soil org. matter by soil micro-organisms, as measured by production of CO_2, was stimulated by addition of NH_4NO_3. P also increased the rate of decomposition but neither element affected the extent of the decomposition. No specific inhibitors of microbial action were detected in soil.

II. Addition of $MgSO_4$ to provide various levels of S in soil (0·32—1·6 mg. of S/100 g. of soil) increased the rate of oxidation of glucose; max. respiration was associated with a C/S ratio of ~900 Other than N and P, S was the only mineral nutrient tested which influenced the microbial activity of soil. $Na_2S_2O_3$, cysteine, cystine, methionine, thioglycollic acid, glutathione and thiamine served as S sources for the micro-organisms. A. G. POLLARD.

Propagation and nitrogen-fixation activity of *Azotobacter* in certain types of soils. V. F. Nepomiluev and L. L. Shishov (*Mikrobiologiya*, 1962, **31**, 294—300).—Experiments showed that in (*a*) turf-podsolic soils with acid reaction and low org. nutrient content *Azotobacter* (**I**) was not widely distributed and occurred in inactive forms with feeble N-fixation powers, >3·6 mg. of N per 1 g. of oxidised sugar. After ploughing-in the turf the organisms increased in no. and N-fixing power. (*b*) In turf gleys **I** were widespread with high N-fixing power. Neutral lowland soils, highly saturated with bases, had **I** widely distributed in humus accumulating layers. Unfavourable physico-chemical and chemical properties in gley-levels of these soils led to absence of **I**. (*c*) In black earth soils the spread and activity of **I** were determined by soil type, nature of crops and cultural operations. W. P. B. HARRISON.

Denitrification in some tropical soils. D. J. Greenland (*J. agric. Sci.*, 1962, **58**, 227—233).—In near-neutral soils held at 70% water-holding capacity, loss of NO_3^- is insignificant, but at 160% water-holding capacity large and rapid loss of added NO_3^- occurs. Soils from under standing forest and old grassland denitrify without the addition of glucose, but a small addition of glucose is required before loss occurs with cultivated or young grassland. This amount is much less than that required on temperate soils. Nitrification and denitrification can take place simultaneously. M. LONG.

Death of root-nodule bacteria on drying. J. M. Vincent, J. A. Thompson and K. O. Donovan (*Aust. J. agric. Res.*, 1962, **13**, 258—270).—Cells of *Rhizobium trifolii* lost viability rapidly when suspended in glass-distilled water and spread on glass beads, under both drying (0 and 20% R.H.) and non-drying (100% R.H.) conditions. Suspension in 9% maltose, although without effect on rate of water-loss, reduced the death-rate considerably during drying and storage at low R.H. As a protectant maltose was markedly superior to yeast extract, sorbitol and five other sugars, including its β-isomer, cellobiose. These materials were, however, superior to water alone, in contrast to NaCl and Ringers solution (equimolar with maltose), which gave no protection. Low maltose concn. (0·9 and 3·6%) were inferior to higher concn. (9, 18 and 27%). The death rate during the drying of cells in water appeared to be inversely related to the concn. of inoculum; maltose removed any such dependence on inoculum size. Under conditions where survival during storage was measurable (maltose and glass beads), 60% R.H. was inferior to 0 and 20%. (10 references.) E. G. BRICKELL.

Seasonal distribution of nutrients in soil under tobacco culture. C. B. McCants (*Soil Sci.*, 1962, **94**, 36—43).—In soils prepared for growth of tobacco transplants changes in soil pH and in the distribution of N, K, Ca, Mg and P resulting from different rainfall patterns are examined. With rainfall <6 in., fairly evenly spread over seven weeks after the planting-out, N and K did not move below 4 in. from the depth of application of fertiliser, leaching losses were small, crop growth was favoured and nutrient intake was correspondingly increased. When 2 in. of rain fell in a 2—3 day period, much K and N was leached, but such conditions did not affect the movement of Mg, Ca or P to >4 in. below the point of application. A. G. POLLARD.

Effects of annual burning on central Missouri prairie. C. L. Kucera and J. H. Ehrenreich (*Ecology*, 1962, **43**, 334—336).—Increased production of dry matter of prairie grasses following burning-over are attributed to earlier growth and less shading in early spring and greater availability of mineral nutrients. The % of nutrient elements in the grass ash showed notable variations with advancing growth but no consistently significant differences between burned and unburned areas were apparent. The ash of accumulated surface litter was greater than in current growth and tended to increase in SiO_2 content as it weathered. A. G. POLLARD.

Comparison of different methods of routine soil analysis. P. A. Gallagher, P. F. Ryan and J. C. Brogan (*Irish J. agric. Res.*, 1961, **1**, 1—5).—The Peech–English modification of Morgan's method and five other methods of testing soils are compared with results of fertiliser trials with a no. of crops. Preliminary data recorded indicate a fair general agreement between methods on a broad basis but more detailed investigations are needed to study the influence of local factors, e.g., type and physical and chemical properties of the soils. A. G. POLLARD.

Perrin–Wilson method for determining phosphorus in fertilisers: delimitation of optimal conditions. T. Niedermaier (*Z. anal. Chem.*, 1962, **187**, 415—424).—Available P_2O_5 is determined by dissolution of the sample in NH_4 citrate solution and pptn. as quinolinium phosphomolybdate. Too large an excess of citric acid delays pptn. and a modified procedure is described which overcomes this difficulty and gives reproducible results for P_2O_5, about 101% of those given by Lorenz's method. J. P. STERN.

Drying and storage of NPK granular fertilisers based on superphosphate and containing urea as a plant nutrient. R. E. Jewell (*J. Sci. Fd Agric.*, 1962, **13**, 414—422).—To ensure stability during storage of fertilisers (N 10, P_2O_5 10, K_2O 18%) containing ammoniated superphosphate and urea (2—15%) a max. granule-drying temp. of 70° is preferable, but if the drying time is short (15 min.) temp. could increase to 90°. Losses were severe at 80—90° in mixtures containing urea and NH_4NO_3 and relatively small when the granular material contained $(NH_4)_2SO_4$. The fertilisers should be stored at temp. >35°. E. M. J.

Use of the water-insoluble product of reaction between phosphoryl chloride and ammonia as a slowly-available fertiliser. S. Ueki and T. Kakizaki (*Rep. Tokyo chem. industr. Res. Inst.*, 1962, **57**, 137—141).—The substance phosphoramide was formed by (1) dropping liquid $POCl_3$ into liquid NH_3 at fairly low temp. and then heating the product above 150°; (2) introducing gaseous NH_3 into liquid $POCl_3$ without cooling, and (3) mixing NH_3 and $POCl_3$ gases. The product obtained by method (1) contained NH_4Cl. Those from (2) and (3) hydrolysed very slowly in water at 30° to produce NH_3 and H_3PO_4. Tests showed that they were suitable fertilisers for paddy rice plants. (From English summary.) O. M. WHITTON.

Micronutrient sources, metal ammonium phosphates as fertilisers. G. L. Bridger, M. L. Salutsky and R. W. Starostka (*J. agric. Fd Chem.*, 1962, **10**, 181—188).—The use of Mg, Fe, Zn, Cu and Mn ammonium phosphates, normally regarded as water insol., as fertilisers and as sources of trace elements with particular reference to $MgNH_4PO_4,H_2O$ (**I**) is examined. Under most conditions nitrification rather than solubility would be the dominant factor in controlling availability of **I** to plants. Response to (**I**) as a source of N and PO_4^{3-} was good; heavy applications, sufficient for 2 years, may be made without risk. In laboratory experiments, seeds were germinated and grown for up to 11 weeks in the pure material. Foliar application of aq. $FeNH_4PO_4,H_2O$ gave a quick response in many cases without the risk of burning associated with the application of chelates. Uptake of Mn from applications of $MnNH_4PO_4,H_2O$ was not affected by liming. A similar behaviour was observed with $CuNH_4PO_4,H_2O$. These phosphates may be incorporated in compound fertilisers without caking problems, and the rate of availability may be controlled by granulation. (18 references.) W. ELSTOW.

Properties and use of micronutrient glasses in crop production. E. R. Holden, N. R. Page and J. I. Wear (*J. agric. Fd Chem.*, 1962, **10**, 188—192).—Micronutrient glasses provide a steady release source of micronutrients without marked seasonal fluctuations. In the case of B deficiency in sandy soils a single application of borax sufficient to survive leaching through the growing season could prove toxic. By the use of B glass in such a case a steady supply of B can be maintained throughout the season. Micronutrient glasses containing a combination of Fe, Mn, Cu, Zn, Mo and B are commercially available in the U.S.A. (30 references.) W. ELSTOW.

Residual effects of phosphorus fertilisers on yields of arable crops; preliminary results of six rotation experiments. H. D. Patterson and R. Williams (*Exp. Husbandry*, 1962, No. 8, 85—103).—Comparison is made of the residual effects of superphosphate, rock P, basic slag and $CaHPO_4$ on potatoes, barley, swedes, kale and a ley over a 7-year period, (*a*) after a single application and (*b*) when the application was repeated at intervals. Interim indications of the results are discussed. A. G. POLLARD.

Manurial value of maize stover. A. G. H. Rattray (*Rhod. agric. J.*, 1961, **58**, 350—353).—Ploughing under maize stover each year was as effective in increasing the following crop of maize as was the application of kraal compost (2 tons per acre). Stubble mulching of maize stover resulted in reduced yields as compared with removal of stover and also increased stalk borer and disease attack. A. H. CORNFIELD.

Fertiliser compound. S.I.L.E. Società Italiana Leucite per Azioni (B.P. 875,543, 7.10.59. It., 7.10.58).—A fertiliser is produced by treating waste material, e.g., leather or wool, with steam at 8—15 atm., then stirring the resulting semi-liquid and concentrating it by cutting off the steam supply and heating until the water content is 2—10%. The product is cooled, then crushed to a powder (or extruded into tablets). F. R. BASFORD.

Temperature control process for making urea–formaldehyde resin fertilisers. Hercules Powder Co. (Inventor: J. M. O'Donnell) (B.P. 875,907, 23.9.59).—A solid urea–CH_2O fertiliser is produced by acidifying to pH 2—4 an alkaline liquid mixture of CH_2O (1) and urea (>1 mol.) to catalyse polymerisation; immediately introducing the mixture on to a continuous, elongated moving surface (to promote formation of a resin layer >0·5 in. in thickness); keeping

the layer at 40—58° until reaction is complete; then heating at ~100° (to remove residual water). F. R. BASFORD.

Phosphatic fertilisers. Fisons Ltd. (Inventors: G. G. Brown and T. P. Dee) (B.P. 873,578, 28.9.58).—In a process for making phosphatic fertilisers from phosphate rock—which yields products of higher solubility in water, and permits higher recovery of P_2O_5—the rock is dissolved in HNO_3 (of concn. 40—60%); H_2SO_4 (of concn. 50—100%) is added to ppt. the Ca as sulphate; the ppt. is removed; further rock is added to the separated liquor in amount to neutralise some of all of the H_3PO_4 present; and the product is finally neutralised (with 25—33% aq. NH_3). The HNO_3 may be replaced wholly or partly by urea nitrate. H. L. WHITEHEAD.

Phosphatic fertilisers. Fisons Fertilizers Ltd. (Inventor: T. P. Dee) (B.P. 876,563, 5.10.56).—Phosphate rock is treated with H_3PO_4 (50—70%) $\leqslant 4$ (4·7) and HNO_3 (40—60%) $\leqslant 4$ (4·7) mol. per mol. of P_2O_5 in the rock and the resulting slurry is treated, under agitation, with a neutralising agent (NH_3 4·7 mol. per mol. of P_2O_5 in the rock). The HNO_3 may be replaced wholly or in part by urea nitrate. J. M. JACOBS.

Phosphatic fertiliser materials. Fisons Fertilizers Ltd. (Inventors: [A, B] J. K. Bradley and W. F. Sheldrick, [A] T. P. Dee, [B] G. G. Brown) (B.P. 876,564—5, 10.1.57).—[A] KCl is caused to react at >80° with a 20—200% excess of HNO_3 over the requirement of 4 mol. of HNO_3 per 3 mol. of KCl to produce a liquid product containing free HNO_3. The NOCl and Cl_2 produced are removed and the liquid product is treated with ground (Ca) phosphate rock. The resulting product is neutralised (with NH_3). The gases containing NOCl and Cl_2 are treated to decompose the NOCl to oxides of N_2 and Cl_2. The Cl_2 is isolated and the oxides of N_2 are recycled to the first stage of the process. [B] The liquid product (after removal of NOCl and Cl_2) is treated with H_3PO_4 before and/or after treatment with a neutralisation agent (NH_3). J. M. JACOBS.

Plant Physiology, Nutrition and Biochemistry

Nitrogen fixation by blue-green algae. III. Growth and nitrogen fixation in *Chlorogloea fritschii*, Mitra. P. Fay and G. E. Fogg (*Arch. Mikrobiol.*, 1962, **42,** 310—321).—Fixation of N by the algae was optimal at 35° and was closely connected with the photosynthetic activity. Very little N was fixed in darkness. A. G. POLLARD.

Changes of amino-acids content of sunflower leaf in the course of 24 hours. T. A. Andreeva and G. F. Korzheva (*Dokl. Akad. Nauk SSSR*, 1962, **143,** 1455—1458).—A study was made of free amino-acids and of protein amino-acids in sunflower leaves, at vegetative and flowering stages. Leaves were sampled at 3-h. intervals when kept over a 24-h. period with natural light or up to 36 h. in darkness Tables show the changes in content of 14 amino-acids. Diurnal variations found in the intensity of photosynthesis in leaves do not cause similar changes in total amino-acid content. Photosynthesis changes the quant. composition of amino-acids and leads to changes in N-exchange processes in leaves. Qual. and quant. composition of protein is the same whether leaves are kept in natural light or darkness, indicating the stability of constitutional protein. (14 references.) P. W. B. HARRISON.

Regeneration in *Cichorium endivia*, L.: endogenous auxins and kinins. M. Vardjan and J. P. Nitsch (*Bull. Soc. bot. Fr.*, 1961, [1962], **108,** 363—374).—Two growth-substances are isolated from chicory roots, (*a*) possibly tryptophan and (*b*) an auxin differing from indolylacetic acid. The distribution of endogenous auxins and kinins during the regeneration of pieces of chicory root is examined. A. G. POLLARD.

Enzymic destruction of ^{14}C-labelled indolylacetic acid and naphthylacetic acid by developing apple and peach seeds. C. W. Donoho, jun., A. E. Mitchell and H. M. Sell (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 43—49).—Seed from developing apple and peach fruit was more effective in destroying indolylacetic acid (IAA) than naphthylacetic acid. The IAA-destroying power of the seed varied considerably with stage of development. A. H. CORNFIELD.

Biological evaluation of 1-naphthyl *N*-methylcarbamate with special reference to the abscission of apple fruits. M. J. Bukovac and A. E. Mitchell (*Proc. Amer. Soc. hort Sci.*, 1962, **80,** 1—10).—When 1-naphthyl *N*-methylcarbamate (NMC) (2 lb. per 100 gal.) was applied at the petal fall and first-cover stages, fruit abscission in McIntosh Northern Spy apples was greater than when Guthion was used. No marked abscission of fruit occurred, except for McIntosh, when NMC was incorporated in the second and subsequent cover sprays. NMC inhibited the elongation of *Avena* coleoptile sections and buckwheat and cucumber roots at concn. $\leqslant 5 \times 10^{-4}$M. Coleoptile elongation was completely inhibited at 10^{-3}M and buckwheat and cucumber roots at 10^{-2}M. 1-Naphthol, a hydrolysis product of NMC, possessed similar inhibitory effects to NMC on coleoptile extension and buckwheat root elongation. A. H. CORNFIELD.

Physiological action of cycloheximide and its behaviour in plant tissue. M. J. Starzyk (*Dissert. Abstr.*, 1962, **22,** 2937).—The systemic activity of cycloheximide in plants and the behaviour of cycloheximide residues on cherry to leaf spot control were studied. Cycloheximide residues on bean leaves are influenced by the period of moisture the leaves received subsequent to spraying. This absorption of cycloheximide may not be influenced by a metabolic process since it occurs at temp. which render most enzymes inactive F. C. SUTTON.

Absorption of maleic hydrazide by citrus. C. H. Hendershott (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 237—240).—Duncan grapefruit and Pineapple orange tree leaves absorbed maleic hydrazide, applied at 2000 p.p.m. (aq. diethanolamine salt plus wetting agent), at similar rates. Valencia orange leaves absorbed maleic hydrazide at a greater rate in R.H. 90—95% than in R.H. 60—70%. Absorption of the chemical was greater from the lower than from the upper surface of the leaf. A. H. CORNFIELD.

Effects of injecting 2,3,5-tri-iodobenzoic acid into *Kalenchoe daigremontiana*. J. Bohlen and H. G. Applegate (*New Phytol.*, 1962, **61,** 132—137).—Injection of TIBA (2×10^{-2}—5×10^{-3} mg.) into leaves of *K. daigremontiana* caused hyponasty; dosages of 0·1 mg. induced epinasty. Changes in O_2 uptake occurred simultaneously and varied directly with the concn. of TIBA used The acid moved upwards and downwards from the point of injection; there was very little lateral movement. A. G. POLLARD.

Formation of abnormal parts in the perianth of flowers of *Tropaeolum majus*, L. and *Linaria spuria*, Mill. during experimental induction by 2,4-dichlorophenoxyacetic acid. P. Dupuy (*Bull. Soc. bot. Fr.*, 1961, [1962], **108,** 375—387).—Modifications of flower structure following applications of 2,4-D are described. Zygomorphic flowers become actinomorphic. A. G. POLLARD.

Induction of parthenocarpy and an attempt to promote apomixis in the strawberry by treatment with growth-regulators. W. J. Lord and D. G. White (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 350—362).—Twenty-nine plant growth-regulators were applied in various ways to five varieties of strawberry in greenhouse tests. Parthenocarpy was induced most frequently by naphthylacetamide (**I**) and indolylbutyric acid applied alone and as mixtures in lanolin smears. Of 11 materials applied in aq. solution only **I** induced parthenocarpy without injury. Berries obtained from blossoms which had been treated were ordinary except that many of the berries were small and achenes were usually small and devoid of embryos. Various growth responses, other than parthenocarpy, which occurred as a result of the treatments are described. A. H. CORNFIELD.

Induction of parthenocarpy in the apple with gibberellin, and the effects of supplementary auxin application. F. G. Dennis, jun. and L. J. Edgerton (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 58—63).—Paste applications of gibberellic acid (5000 p.p.m.) as K salt to emasculated McIntosh apple blossoms were the most effective of a no. of methods of application in terms of initial fruit set, but final yields were reduced regardless of method of application. The seedless fruit obtained was smaller than open-pollinated fruit. Most of the parthenocarpic fruitlets abscissed during the June drop. Of a no. of auxins tested, 2-chloro-4-fluorophenoxyacetic acid was the most effective in reducing abscission, but it inhibited fruit growth. A. H. CORNFIELD.

Effects of gibberellic acid on growth and anatomy of some horticultural crops. J. M. Bostrack (*Dissert. Abstr.*, 1962, **22,** 2955).—Different effects of gibberellic acid (GA) on different varieties and on various plant parts are described. Although the % dry wt. of the fresh wt. was not significantly different it tended to be slightly higher in plants treated with GA in all cases. F. C. SUTTON.

Effect of gibberellic acid on cherry seedling growth. S. W. Zagaja (*Hort. Res.*, 1962, **1,** 81—84).—Daily treatment with gibberellic acid of dormant cherry seedlings with normal and crinkled leaves produced from partially after-ripened embryos resulted in a significant increase in shoot growth but had no effect upon the abnormal (crinkled) leaves. J. L. WALPOLE.

Effect of gibberellic acid on the germination, growth and development of *Trifolium repens*, L. W. W. Fletcher and D. J. Martin (*J. agric. Sci.*, 1962, **58,** 235—241).—Gibberellic acid did not affect the germination of six small-seeded varieties of legumes tested. All varieties of *T. repens*, L. tested showed an increase in petiole length and individual leaf area and a decrease in stolon no. and length. Total leaf area remained unchanged, but shoot and root dry wt. were reduced. The stolons became negatively geotropic. Since treated plants of wild white clover resemble untreated plants

of ladino clover, endogenous levels of gibberellins may play an important part in the development of varieties. M. LONG.

Effect of gibberellic acid on circumnutation. A. J. McComb (*New Phytol.*, 1962, **61,** 128—131).—Treatment of pea seedlings with gibberellic acid (5 μg. in 4 μl. droplet in 50% ethanol) applied to a leaflet increased the amplitude of circumnutation. The mechanism of this action is discussed. A. G. POLLARD.

Natural synergists of auxins and gibberellins. J. P. Nitsch and C. Nitsch (*Bull. Soc. bot. Fr.*, 1961, [1962], **108,** 349—362).—A mathematical expression of the phenomenon of synergism is presented. Active synergists of indolylacetic acid (**I**) include chlorogenic and isochlorogenic acids, protocatechnic acid, glutathione, quercitin and derivatives, kaempferol, α-tocopherol and vitamin K_1. *trans-p*-Coumaric acid, naringenin, naringin, coumarin and scopoletin had marked inhibitory effects. Synergists of **I** had no corresponding effects on 1-naphthylacetic acid or gibberellin.
A. G. POLLARD.

Crops and Cropping

Dormancy in wheat. B. Belderok (*Proc. int. Seed Testing Ass.*, 1961, **26,** 697—760, Reprint).—A review covering sprouting in the ear, analysis of, and causes of dormancy. Dormancy in wheat might be associated with the existence of disulphides in the covering layer. A close correlation exists between the degree of dormancy and the contents of bound disulphide linkages in the covering layers of the grains. (90 references.) E. M. J.

Influence of variations in harvesting and initial storage on wheat kept for several years. F. S. Mitchell and F. Y. K. Caldwell (*J. agric. Engng Res.*, 1962, **7,** 27—41).—Effects of harvest moisture content and drum setting were investigated by testing germination of dried sack-stored wheat over 64 months. Grain harvested at <19% moisture germinated well over 5 years; that harvested at >25% moisture showed greatly reduced germination and marked mould development. Variation in drum setting had the greatest effect in the harvest moisture content range 19—25%.
A. H. CORNFIELD.

Copper and nitrogen in the nutrition of wheat on cutaway peat. G. A. Fleming and J. Delaney (*Irish J. agric Res.*, 1961, **1,** 81—82).—Cu deficiency in wheat on these soils was intensified by application of N fertiliser ($CaNH_4$ nitrate). The effect was corrected by $CuSO_4$ at the rate of 7 lb. per acre applied 2 years previously.
A. G. POLLARD.

Effect of variety and depth of sowing on depth of secondary root zone in oats. P. L. Rodgers (*Proc. Iowa Acad. Sci.*, 1961, **68,** 124—128).—Depth of initiation of secondary roots was related to depth of sowing but was independent of variety. E. G. BRICKELL.

Molybdenum deficiency in maize. J. J. Du Toit (*S. Afr. J. agric. Sci.*, 1962, **5,** 127—132).—By planting seed that had been soaked for 8 h. in solutions containing 0·01% of Mn, Zn, Fe, B, Mo and Cu both singly and in combinations of five of these elements at a time, a case of severe yellowing and stunted growth of maize was shown to be due to Mo deficiency. Both Mn and Zn had a definite depressing effect on growth and yield. Application to the seed of 6·9 g. of NH_4 molybdate per acre increased the yield of dry grain by 737 lb. per acre. (In Afrikaans.) W. ELSTOW.

Assessing potato damage resulting from mechanical handling. J. S. Aspinwall, R. Q. Hepherd and P. Hebblethwaite (*J. agric. Engng Res.*, 1962, **7,** 71—72).—Samples of potatoes from the harvester are dipped in aq. I_2–KI containing methyl cellulose and Saturn Yellow (fluorescent tracer). Damage due to harvesting is thus assessed. After drying the treated potatoes are mixed with the rest of the batch and put through the mechanical handling process. The marked tubers are picked out by use of u.v. light and dipped into *p*-cresol to show damage caused by the handling operation. A. H. CORNFIELD.

Modifications of the chemical composition of potatoes due to changes observed in crops on recently reclaimed land. W. Routchenko (*C. R. Acad. Agric. Fr.*, 1962, **48,** 371—376).—The incidence of black heart in potatoes on moorland soil is attributed to the rapid seasonal changes in soil-water levels observed in these locations; high-lying parts become rapidly dried up whilst low-lying parts become waterlogged in spring, causing the crops to suffer from cold and soil-O_2 deficiency. Remedial measures include improvement of the soil to secure adequate drainage and aeration when waterlogged, irrigation of the soil when excessively dry, increasing the soil-pH and, if possible, the selection of resistant varieties. (16 references.) P. S. ARUP.

Field experiments on the magnesium requirement of potatoes in Great Britain. M. R. J. Holmes (*J. agric. Sci.*, 1962, **58,** 281—285).—An appreciable response to Mg can be obtained with potatoes on sands and sandy loams; exchangeable Mg does not generally indicate the response to be expected. High K rates depress yields, but do not generally induce Mg deficiency. M. LONG.

Response of sugar beet to fertiliser and the effect of farmyard manure [FYM]. S. N. Adams (*J. agric. Sci.*, 1962, **58,** 219—226).—Optimal dressings for sugar yield without FYM are N 1·0, P_2O_5 0·5 and K_2O 1·6 cwt. per acre. Where 12 tons of FYM per acre are applied, the respective dressings are N 0·6, P_2O_5 0·0 and K_2O 0·8 cwt. N response varies from field to field, past cropping being the best guide, although where beet follows cereal, more N is required. Soil analysis serves only to indicate fields requiring more than the average P_2O_5 dressing. M. LONG.

Effects of heavy application of nitrogen on the composition of herbage. E. Conroy (*Irish J. agric. Res.*, 1961, **1,** 67—71).—The herbage (Cockle Park mixture) on an acid loam to which large applications of $(NH_4)_2SO_4$ (up to 16 cwt./acre) had been given showed increased contents of S (of which 60% was present as SO_4^{2-}), Mn and NO_3^-, the last named reaching toxic levels, adversely affecting palatability and causing scouring in July—Aug.
A. G. POLLARD.

Effects of mid-spring applications of nitrogen on an irrigated pasture. J. Lammerink (*N.Z. J. agric. Res.*, 1962, **5,** 101—110).—Herbage production (associations of grasses and clovers) can be substantially increased over the mid-spring period by a single application of an NH_4NO_3–lime mixture (3 cwt./acre). When the application was divided into six fortnightly dressings, the response was spread over a longer period. E. M. J.

Liquid manure as a grassland fertiliser. I. Response to liquid manure and to dry fertiliser. M. E. Castle and A. D. Drysdale (*J. agric. Sci.*, 1962, **58,** 165—171).—Plain water (**I**) treatments affect neither herbage dry matter nor crude protein yields, whilst liquid manure (**II**) treatments increase both. Applied P has no effect on plots treated with **II**. The crude protein content of the herbage is not significantly affected by either **I**, **II** or dry fertiliser (**III**) treatments. Clover increases with **II**, whilst meadow fescue and dicotyledonous weeds decrease. The pH and P content of the soil is unaffected by any of the treatments. **II** and **III** both increase the K content of the soil. M. LONG.

Effects of different dates of grazing and nitrogen top-dressing on the subsequent hay crop. J. C. Wilcox (*Exp. Husbandry*, 1962, No. 8, 104—112).—Yields of hay (dry matter) were lowered by 3·3 cwt./acre by spring grazing of sheep from Feb. to late March or early April. Further losses were incurred by permitting grazing until mid-May. Nitrochalk, applied after grazing, increased dry matter yields and (slightly) the % protein, but had no appreciable effect on the % sugar or crude fibre in the hay. Grazing treatments did not affect the % sugar, protein or crude fibre. The proportion of clover in the herbage was increased by grazing early or both late and early but was lowered by the N dressing.
A. G. POLLARD.

Crop conditioning. I. Drying rates and yields. G. Shepperson, J. K. Grundey and R. Wickens (*Exp. Husbandry*, 1962, No. 8, 65—84).—Data showing the effects of various harvesting implements or sequence of implements and subsequent treatments on the drying rate and final yield of pasture herbage are recorded.
A. G. POLLARD.

Establishment of grass–clover swards on bogland by surface seeding. D. P. Coltins (*Irish J. agric. Res.*, 1961, **1,** 21—30).—Investigations of surface seeding (lime, fertiliser and seed applied directly on the existing vegetation) on an acid peat (pH 4·7) soil are described. To establish a grass–clover sward applications of Ca and P were essential. K favoured growth of clover and increased yields of herbage; Mo increased and N restricted clover growth. As P source in presence of lime, superphosphate was superior to basic slag. The action of ground mineral P was lower in presence of lime or of heavy application of basic slag. A. G. POLLARD.

Comparison of potassium chloride, bicarbonate and metaphosphate and calcined orthoclase, as sources of potassium for white clover. A. J. Metson and W. M. H. Saunders (*N.Z. J. agric. Res.*, 1962, **5,** 145—157).—Pot tests with a K-deficient yellow-brown loam showed that $KHCO_3$ and KPO_3 were at least as effective as KCl as measured by yield of dry matter and uptake of K. No indication of a longer duration of response from $KHCO_3$ and KPO_3 as compared with that from KCl was obtained. Percolation losses of K from all treatments were small even at the higher rates of application. Calcined feldspar was evaluated on an acid infertile peat soil. Neither heat treatment nor fine grinding would produce a low-K material with K made sol., but less susceptible to luxury consumption than that in KCl. (22 references.) E. M. J.

Magnesium in forage plants. III. Magnesium distribution in pastures of low magnesium content. J. R. Todd (*J. agric. Sci.*, 1962, **58**, 277—279).—In pasture of low Mg content more than 50% of the total Mg is water-sol.; insol. Mg amounting to $\frac{1}{3}$ of the total in young leafy pasture and $\frac{1}{5}$ to $\frac{1}{4}$ in pasture at the seedling stage. Acetone-sol. Mg varies from 12% of total in young leafy grass to 5% in seedling grass. Fertiliser treatments do not alter the relative amounts of fractions. M. LONG.

Influence of rootstock, bodystock and interstock on the nutrient content of apple foliage. R. B. Tukey, R. Langston and R. A. Cline (*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 73—78).—The concn. of major elements (N, P, K, Ca and Mg) in the leaves of bearing apple trees varied markedly depending on rootstock, bodystock, interstock and scion and the no. of component parts grafted together. The effects varied with season and soil type. All these factors should be considered when leaf analysis is used as a diagnostic aid for indicating fertiliser requirements of apple trees. A. H. CORNFIELD.

Influence of oxygen and carbon dioxide on the development of apple scald. M. E. Patterson and M. Workman (*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 130—136).—Storage of a scald-susceptible apple variety at 0° for 114 days resulted in no scald when the O_2 concn. of the atm. was 1%, but a high incidence of scald with 4% or more O_2 in the atm. Scald incidence decreased with increasing concn. of CO_2 in the atm. up to 18%. Softening was retarded more by low O_2 concn. than by high CO_2 concn. The presence of 600 p.p.m. of C_2H_4 in the atm. had no effect on scald development or ripening during storage. A. H. CORNFIELD.

Effects of early-season sprays of boron on fruit set, colour, finish and storage life of apples. W. J. Bramlage and A. H. Thompson (*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 64—72).—Application of 'Solubor' (78% $Na_2B_8O_{13},4H_2O$–20% $Na_2B_4O_7,5H_2O$, 1 lb. per 100 gal.) at full bloom and early petal-fall to apple trees increased, temporarily, the B concn. in the leaves. The increased B concn. in the fruit persisted throughout the season. Fruit set was increased in only one of three varieties. The treatments retarded the development of Jonathan spot, but had no effect on fruit colour or finish, decay or scald during storage, or firmness, moisture content, total sugars and alcohol-insol. solids. A. H. CORNFIELD.

Effect of spray additives and simulated rainwater on foliage curvature and thinning of apples by the sodium salt of naphthylacetic acid. F. Horsfall, jun. and R. C. Moore (*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 15—32).—The NH_4 salts of some relatively strong inorg. and org. acids, applied at 256 p.p.m. NH_3, increased the absorption of naphthylacetic acid Na salt (**I**), as measured by increased angle of curvature of the apple leaf. CO_3^{2-} and HCO_3^- of NH_4^- and Na_2SO_4 inhibited the effect of **I**. $(NH_4)_2SO_4$ sprays (2—32 p.p.m. NH_3) applied before applications of **I** increased the absorption of the latter in greenhouse tests. In orchards, application of soft waters containing $(NH_4)_2SO_4$ before the application of **I** increased the fruit-thinning action of the latter. A no. of pesticides and spray adjuvants increased the action of **I**. A. H. CORNFIELD.

Yield, fruit size and growth of York Imperial apple trees as affected by chemical thinning and differential nitrogen nutrition for six years. B. L. Rogers and A. H. Thompson (*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 50—57).—Application of naphthylacetamide (25—50 p.p.m.) at the post-bloom stage over 6 years commencing at 18 years of age increased fruit size and trunk growth, had no effect on fruit yields, and decreased terminal growth. Extra N fertiliser increased leaf-N and terminal growth, but had no effect on fruit size or yield. A. H. CORNFIELD.

Influence of chemical thinners on the growth rate of apples. F. W. Southwick, W. D. Weeks, E. Sawada, and J. F. Anderson (*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 33—42).—Application of 10 p.p.m. naphthylacetic acid (NAA) 18—22 days after petal-fall had a direct temporary size-inhibiting action on the growth rate of persisting Golden Delicious and Early McIntosh apples. Similar applications of 1-naphthyl *N*-methylcarbamate (NMC) (1·5 lb. per 100 gal.) did not influence the growth rate of persisting fruit. NMC at 1—2 lb. per 100 gal. usually had a milder thinning action and was less injurious to apple foliage than were NAA and naphthaleneacetamide at standard rates. NMC failed to thin one variety after petal-fall. A. H. CORNFIELD.

Effect of 1-naphthyl *N*-methylcarbamate (Sevin) as a chemical thinner for apples in Western Colorado. R. L. Stebbins (*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 11—14).—Application of Sevin (1—2 lb. 50% wettable powder per 100 gal.) 7 days and 14 days after full bloom thinned four species of apple. Later applications resulted in less effective thinning. A. H. CORNFIELD.

Chelates of iron in treatment of chlorosis of pear tree. R. Guennel on (*C.R. Acad. Agric. Fr.*, 1962, **48**, 209—213).—Chlorosis due to excessive soil-Ca (soil-pH 8·1) can be controlled by applications of the Fe chelate of the Na_3 salt of ethylenediaminedi-*o*-hydroxyphenylacetic acid. The best results were obtained by applications of the commercial prep. containing 15 g. of active material applied in spring to the soil round each tree. Good results were obtained with the free acid, and with a prep. based on the Fe-NH_4 salt of the amide of the acid. P. S. ARUP.

Influence of the morphology of raspberry canes on their liability to be attacked by certain fungi. D. L. Jennings (*Hort. Res.*, 1962, **1**, 100—111).—Observations have been made of the incidence of three fungal diseases of raspberry seedlings—spur blight (*Didymella applanata*), grey mould (*Botrytis cinerea*) and cane spot (*Elsinoe veneta*)—in plant breeding trials in which the morphological characteristics of hairiness, spininess and the thickness of the wax coating were selected. No consistently positive correlations were found but there is evidence that these characteristics enable the cane surfaces to avoid infections. Alternative hypotheses based on the inheritance of certain genes are discussed. (17 references.) J. L. WALPOLE.

Anatomical and hormonal development in Redhaven peach seeds as related to the timing of naphthylacetic acid for fruit thinning. P. B. Lombard and A. E. Mitchell (*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 163—171).—Application of 30 p.p.m. of naphthylacetic acid (NAA) sprays to Redhaven peach trees gave significant fruit thinning when the spray was applied 35 or 42 days after bloom, but not when applied 49 days after bloom. Thinning with NAA was related to change of the embryo from the filamentous to the spherical form, to cytokinesis of the endosperm, and to a high level of natural hormones in the seed. A. H. CORNFIELD.

Light transmission for assessing the maturity of peaches, nectarines and plums. R. J. Romani, F. C. Jacob and C. M. Sprock (*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 220—229).—The ratio of light transmission through the fruit at approx. 730 and 850 mμ was useful in following the extent of maturity of eight varieties of peach and seven of nectarine. Results with plums were not as satisfactory, although changes in firmness were correlated with differences in peak transmission at 580 and 620 mμ over one month of picking. A. H. CORNFIELD.

Effect of combinations of urea–formaldehyde, urea and ammonium nitrate on fruit and shoots of mature peach trees. L. P. Somogyi, N. F. Childers, W. J. Kender and M. J. Prusik (*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 190—196).—Application of 0·5 lb. of N per tree per annum to peaches growing on a sand produced no significant yield differences with one variety irrespective of whether the N was supplied as NH_4NO_3 + urea or urea–formaldehyde. With another variety yields were higher with urea-N than with urea–formaldehyde-N. There was a rough correlation between yields and N% in the fruit. With one variety high N% in the fruit was associated with reduced red colour of the skin. There were no differences between treatments in shoot growth or size, firmness or sol. solids of the fruit in either variety. A. H. CORNFIELD.

Response of orange trees and fruits to freezing temperatures. C. H. Hendershott (*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 247—254).—The cooling rate of the fruit on bearing trees subjected to freezing temp. was not influenced greatly by fruit size in the two varieties of orange studied. The f.p. of orange fruits ranged from −2·5° to −1·7°. Fruit usually subcooled to −4·4° to −3·3°, depending on length of exposure, before freezing. The crit. temp. for leaf killing was −6·6°. Wood damage at freezing temp. was associated with the length of new growth present. A. H. CORNFIELD.

Seasonal changes in the free amino-acids in Valencia orange juice. R. L. Clements and H. V. Leland ((*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 300—307).—Over the 15 months from Sept. to Dec. total N and amino-acid N concn. increased in the filtered juice of Valencia oranges. Proline, particularly, and arginine and γ-aminobutyric acid were present in the highest concn., especially after maturity. Proline increased to a greater extent than did the other amino-acids. NH_3, asparagine, aspartic acid and serine predominated in the juice of the early fruit. Amino-acid-N accounted for 61% of the total N in the juice of the early and 83% in that of the late fruit. A. H. CORNFIELD.

Macroelement composition of orange leaves from non-fruiting and fruiting terminals. R. B. Harding, T. M. Ryan and G. R. Bradford (*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 255—258).—The average % of N, P, K and S (dry basis) in 4–6-month-old orange leaves was significantly higher, whilst the % of Ca, Mg and Cl was significantly lower from non-fruiting than from fruiting terminals. No significant differences occurred with Na. Leaf sampling position is important when leaf analysis is used as a guide to the nutrient status of the tree. A. H. CORNFIELD.

Influence of rootstocks on the concentration of boron manganese, iron and other elements in lemon leaves, and on boron toxicity symptoms. T. W. Embleton, C. K. Labanauskas and W. P. Bitters (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 285—290).—Alemow (*Citrus macrophylla*) rootstocks produced lower concn. of B in the leaves and lower incidence of B-toxicity leaf symptoms with Frost Nucellar Eureka lemon scions than did 8 other rootstocks. Alemow rootstock also produced relatively high leaf-Mn and low leaf-Mg in comparison with the other rootstocks. Yuzu (*C. junos*) rootstock produced higher leaf -Mn, -Fe and -Na, and lower -Ca than did the other rootstocks. Yuzu may be useful where Fe and Mn are likely to be deficient, but not where available Na is likely to be high. A. H. CORNFIELD.

Effects of nitrogen, phosphorus, potassium, limestone, gypsum and manure soil applications on soil pH and on macro- and micronutrient concentrations in Washington navel orange leaves. C. K. Labanauskas, W. W. Jones and T. W. Embleton (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 259—267).—The effects of soil applications of N, P, K, $CaCO_3$, manure and $CaSO_4$ on leaf nutrient concn. were not related to changes in soil pH induced by the treatments, except where $(NH_4)_2SO_4$ (**I**) was applied, where pH was reduced and leaf Mn increased considerably. Applications of $NaNO_3$ which increased soil pH also increased leaf-K, -Na and -B in comparison with applications of $Ca(NO_3)_2$ (**II**). **I** increased leaf-Mg to a greater extent than did **II**. $CaCO_3$ reduced leaf-Mg and -Mn. $CaSO_4$ reduced soil pH and leaf-Na. Superphosphate decreased leaf-Cu. K_2SO_4 increased leaf-K and -Cl. Manure increased leaf-K and decreased leaf-N and -Fe in comparison with leaves of trees treated with similar quantities of inorg. N, P and K. Leaves from trees growing under tillage plus winter clover crop had higher Ca, Mg, Na and B and lower P and K contents than had leaves from trees on chemically weeded plots. A. H. CORNFIELD.

Influence of maleic hydrazide on citrus trees and fruits. C. H. Hendershott (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 241—246).—Growth of a no. of varieties of young citrus trees was stopped by application of maleic hydrazide (1000—2000 p.p.m.) in early Nov. thus giving a few degrees of cold resistance. The length of the dormant period induced by the treatment varied with variety and season from 1 to 7 months. In the spring following treatment long narrow leaves appeared in the first growth, multiple buds and shoots were formed and small, pear-shaped, thick-peeled fruit which was slow in development also resulted. A. H. CORNFIELD.

Potassium, calcium and magnesium in the nutrition of pineapples in Guinea. VII. Conclusions. P. Martin-Prével (*Fruits, Paris*, 1962, **17,** 257—261).—The findings from the previous studies are summarised and discussed in regard to improvement in the cultivation of pineapples (cf. J.S.F.A. Abstr., 1962, ii, 186; i, 210, 125; 1961, ii, 228, 211; 1960, i, 17; 1959, ii, 19). E. M. J.

Application of powdered urea to the foliage of pineapples. C. Py (*Fruits, Paris*, 1962, **17,** 285—287).—Powdered urea (2 or 4 g./plant) is applied to the leaves late in season when N fertiliser in solid form given at the foot of the plant would not be assimilated. It has no effect on the natural or provoked flowering period, but is useful in a dry season. The wt. of leaves and of fruit is increased from 27 to 34%. E. M. J.

Cracking of prunes in relation to irrigation. K. Uriu, C. J. Hansen and J. J. Smith (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 211—219).—Side-cracking of prunes appeared at a definite stage in the development of the fruit, regardless of differences in irrigation treatment. End-cracking occurred immediately after irrigation in plots which had been under soil moisture stress and providing the fruit was not near maturity. Very little end-cracking occurred on trees supplied with adequate moisture throughout the season. A. H. CORNFIELD.

Correction of manganese deficiencies in grapefruit trees by foliar sprays in desert areas of Southern California. C. K. Labanauskas (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 268—273).—Foliar applications of $MnSO_4$ (3 lb. per 100 gal.) in April and Nov. eliminated Mn-deficiency symptoms on sprayed leaves, but not on subsequent growth. Leaf-Mn was increased slightly by the treatment, but leaf-Zn, -Cu, B and -Fe were unaffected. Application of Mn chelate (1 lb. per 100 gal.) did not eliminate Mn-deficiency symptoms or increase leaf-Mn. Leaf and soil applications of Fe chelate did not eliminate Fe-deficiency symptoms or increase leaf-Fe. A. H. CORNFIELD.

Effects of growth regulators on the fruiting of cranberries, ***Vaccinium macrocarpon.*** C. C. Doughty (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 340—349).—None of the seven chemicals tested alone or in combination was satisfactory for increasing yields of cranberries during the season in which the treatments were applied. Application of *p*-chlorophenoxyacetic acid (20 p.p.m.) at 45—55% and again at 80—95% full bloom increased blossom initiation and yields in the following year. Application of 2,4,5-T (2—3 p.p.m.) post-harvest in Oct. increased the no. of fruiting uprights, blossoms per sq. ft. and berry yields in the following year. The effects of the treatments on histological development of blossoms and blossom-buds are also reported. A. H. CORNFIELD.

Sulphate and chloride effects upon Wolcott blueberry growth and composition. W. E. Ballinger (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 331—339).—The blueberry plant tolerated fairly high levels of Cl^- when grown in sand culture. Slight abnormal effects occurred with 104 mequiv. of Cl per l. of nutrient. High Cl^- and low SO_4^{2-} in the nutrient reduced root growth and top wt. Leaf analysis of commercial plantings indicated that Cl^- is not being used in excessive amounts. Harmful effects occurred only when leaf Cl was >0·5% (dry basis). A. H. CORNFIELD.

Seasonal changes in the nutrients in the leaves of Rubel blueberry bushes. J. S. Bailey, A. F. Spelman and B. Gersten (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 327—330).—Over two seasons the levels of N, P, K and Mg in the leaves of Rubel blueberry showed seasonal changes similar to those occurring in late apples and the Elberta peach. Leaf-K in the blueberry showed a late season increase which did not occur in the other species. Leaf sampling just before fruit ripening is probably the most desirable time when all five elements are to be analysed. A. H. CORNFIELD.

Effects of differential applications of nitrogen, potassium, magnesium, boron and phosphorus on their concentration in leaves of filbert trees. J. H. Painter and H. E. Hammar (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 315—326).—Application of the high level of NH_4NO_3 (**I**) increased leaf-Mn over the low level of application, probably due to decreased soil pH resulting in increased available soil Mn. The higher level of KCl application also increased leaf-Mn over the lower level, but not to quite the same extent as did **I.** This treatment did not alter soil pH. Borax (4 lb./tree) was toxic. Leaf-Mg was increased by the high level of $MgSO_4$, reduced by **N** and unaffected by KCl, superphosphate and B applications. Leaf-P was reduced by N and B applications but not affected by superphosphate. Leaf-Ca was usually unaffected by the treatments, except for reductions where toxic amounts of B were applied. Leaf Fe and -Zn were not consistently affected by the treatments. The treatments had no consistent effect on leaf-Cu, except that high N increased leaf-Cu in the last two of the 6 years. A. H. CORNFIELD.

Zinc chelate and zinc sulphate sprays for controlling rosette on Schley and Stuart pecans. A. O. Alben (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 312—314).—Application of Zn-Na_2-EDTA (14% Zn, 0·5—3·0 lb. per 100 gal.) or $ZnSO_4$ (36% Zn, 2 lb. per 100 gal.) on May 10 and 24 resulted in complete recovery of moderately rosetted pecan trees with the higher Zn concn. Severely rosetted trees recovered somewhat, the extent of recovery being roughly proportional to the concn. of Zn applied. The $ZnSO_4$ spray was not as effective as the highest concn. of Zn-EDTA. A. H. CORNFIELD.

Chlorine and other elements in avocado leaves as influenced by rootstock. T. W. Embleton, M. Matsumura, W. B. Storey and M. J. Garber (*Proc. Amer. Soc. hort Sci.*, 1962, **80,** 230—236).—The Guatemalan varieties of rootstocks produced significantly lower Cl in scion leaves of avocado than did the Mexican varieties. There were also significant differences between varieties within races. Rootstock race and variety influenced significantly the N, P, K, Ca and Mg concn. in scion leaves. Leaf nutrient content and variability also varied depending on whether the seedlings were propagated on their own roots from cuttings or were grafted. Results are discussed in relation to tipburn of avocado growing on soils naturally high in Cl. A. H. CORNFIELD.

Case of manganese toxicity affecting a cold-house tomato crop. J. G. D. Lamb (*Irish J. agric. Res.*, 1961, **1,** 17—20).—In an acid soil, not heated or steam-sterilised, the tomato 'Ware Cross' developed symptoms of Mn toxicity when the pH was <5. Leaf chlorosis and failure to set fruit occurred, notably at the level of the fourth and fifth trusses. The disorder was less severe and the Mn content of the leaf tissue was smaller after heavy applications of P fertiliser. A. G. POLLARD.

Some effects of phosphate fertiliser on leaf composition in tomato. J. G. D. Lamb (*Irish J. agric. Res.*, 1961, **1,** 73—80).—On a soil of low available Mg content and high PO_4^{3-}-fixing capacity, applications of superphosphate increased the P and K contents of tomato leaves, lowered those of Mg and Cu and increased the N content of the lower leaves. A. G. POLLARD.

Growth and nutrient absorption of green bunching onions. F. W. Zink (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 430—435).—Growth rate (plant height, leaf no. and fresh wt.) and nutrient content (NO_3^-

total N, P, K, Na, Ca, Mg) during growth of a spring, summer and winter crop of bunching onions are reported. A. H. CORNFIELD.

Respiration and storage deterioration in celery as affected by post-harvest treatments with N^6-benzylaminopurine. S. H. Wittwer, R. R. Dedolph, V. Tuli and D. Gilbart (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 408—416).—A post-harvest dip of celery stalks in 10 p.p.m. N^6-benzylaminopurine extended the duration of visual freshness, green coloration and market acceptibility and reduced respiration losses during storage. A. H. CORNFIELD.

Indoxyl acetate as an indicator of cracked seed coats of white beans and other light-coloured legume seeds. R. C. French, J. A. Thompson and C. H. Kingsolver (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 377—386).—An indoxyl acetate technique for staining cracked seed coats is described. The method is rapid, stains only cracked seed, and the stained area is much larger than the seed coat crack. The staining treatment has no effect on the growth of seedlings from uncracked pea beans. The method was applied successfully to the seed of a no. of species. It was not satisfactory for maize seed. A. H. CORNFIELD.

Effects of singling and advancing season on the composition of thousand-headed kale. D. J. C. Jones (*J. agric. Sci.*, 1962, **58,** 265—276).—The top half of the stem is intermediate in composition between the bottom half and leaf, except for K, which is highest in the top half. The % dry matter increases in all three portions as the season progresses. Singling has no significant effect, although singled crops tend to have lower crude fibre and higher crude protein contents. M. LONG.

Factors affecting response of soya-beans to irrigation. A. L. Matson (*Dissert. Abstr.*, 1962, **22,** 2931—2932).—Affecting factors were studied in three experiments conducted on a sandy loam soil in Missouri. Investigations were made on the period of critical water need; the effects of variety, planting date, P and K fertilisers, and plant spacing on the response to full season irrigation and limited irrigation; and the effects of N fertiliser and the time of application and plant spacing and row width combinations on three soya-bean varieties with and without irrigation. Variations in seed quality, lodging and protein content are recorded. F. C. SUTTON.

Influence of soil and irrigation water on the chemical composition and quality of cigar tobacco. C. K. R. Kurup and A. S. Sastry (*J. Indian Soc. Soil Sci.*, 1962, **10,** 99—108).—Relationships between tobacco leaf composition and burning quality are examined. In two varieties, locality of growth influenced leaf composition but the total cation contents showed little divergence. Variations in the ratios, $K_2O/N + Cl$ and $(K_2O + CaO)/(MgO + N + Cl)$ showed trends corresponding with leaf-burn and cigar-burn tests and probably serve as measures of leaf quality. In irrigation waters of similar SO_4^{-2} content the $[Cl^-]$ largely controls the Cl content of the leaf. A. G. POLLARD.

Development of an improved method for the biological retting of kenaf ribbons. K. J. Scott (*S. Afr. J. agric. Sci.*, 1962, **5,** 133—144).—The plant acids present in kenaf ribbons prevent retting commencing at once on soaking in water. Thus in tank retting a continuous purge of water at 32° is required to keep the pH of the soak liquor >4·5. Under these conditions with a water to ribbon ratio of 20 : 1 retting reaches completion in 4—6 days. Kenaf has the advantage over hemp in that it does not over-ret and lose fibre strength, and therefore tank retting which is faster is just as safe as canal retting for kenaf. An improved method which incorporates the advantage of tank retting and used only 50 lb. of water per lb. of dry fibre is described. W. ELSTOW.

Improvement of pyrethrum yields through the application of fertilisers. U. Kroll (*Pyrethrum Post*, 1962, **6,** No. 3, 32—33).—Increased yields of pyrethrum flowers frequently follow the application of sol. phosphates but N, K and the minor elements have little effect and lime has given variable results. Cultural practices are also of great importance. J. C. WALPOLE.

Factors involved in the vegetative propagation of *Dioscorea spiculiflora* Hemsl. from vines. W. H. Preston, jun. and J. R. Haun (*Proc. Amer. Soc. hort. Sci.*, 1962, **80,** 417—429).—Factors affecting the efficiency of vegetative regeneration from vines of *Dioscorea spiculiflora*, a plant of potential importance because of the steroidal sapogenins in its tuberous roots, were studied. An increase occurred in rooting and sprouting of large vine segments from mature, but not from 1-year-old, plants treated at 100 p.p.m. with α-methoxyphenylacetic acid (Na salt). Treatment with other hormones has little effect. Gibberellic acid (K salt) at 50 p.p.m. almost completely inhibited development of new plants from axillary buds. A. H. CORNFIELD.

Pest Control

European maize borer, green peach aphid and cabbage looper control on peppers. P. P. Burbutis, D. J. Fieldhouse, D. F. Crossan, R. S. VanDenburgh and L. P. Ditman (*J. econ. Ent.*, 1962, **55,** 285—288).—Weekly DDT sprays increased yields and reduced the no. of borers trapped. Populations of *Ostrina nubilalis* were higher on plots receiving a high level of fertiliser. The use of DDT sprays resulted in higher no. of *Myzus persicae*. DDT soil treatment caused a smaller increase and three applications of DDT + thiodan kept them below the control. Weekly DDT sprays also controlled *Trichoplusia ni*. C. M. HARDWICK.

Insecticidal field trials for control of potato aphids in New Brunswick, 1948—60. D. D. Pond (*J. econ. Ent.*, 1962, **55,** 306—308).—The effectiveness of various formulations over 9 years was very variable. Malathion was ineffective with cool temp. Pyrethrum and toxaphene synergised DDT. Sevin was not aphicidal. Diazinon, malathion and Thiodan are currently recommended. In general insecticidal application did not increase yields. C. M. HARDWICK.

Lucerne weevil control studies in West Virginia. C. K. Dorsey and D. O. Quinn (*J. econ. Ent.*, 1962, **55,** 365—368).—Both spring and autumn applications of granular or spray heptachlor at 0·5 or 1·0 lb./acre gave good control of *Hypera postica*. Dieldrin was less satisfactory. Twelve org. P compounds alone or in mixtures gave poor results. (12 references.) C. M. HARDWICK.

Control of the white-fringed weevil, *Graphognathus leucoloma*, Boh. W. E. Wright (*Agric. Gaz. N.S.W.*, 1961, **72,** 631—634).—The pest was controlled in lucerne by application, soon after emergence of adults, of dieldrin (1 lb.), aldrin (2 lb.) or heptachlor (2 lb. per acre in 10 gal. water). In vegetables and ornamentals the pest was controlled by treating the soil at the base of the plants with 0·1% DDT, 0·05% dieldrin or 0·1% aldrin. A. H. CORNFIELD.

Streptomycin–oxytetracycline sprays for control of fire blight, caused by *Erwinia amylovora*, on Bartlett pear. H. L. Keil and R. A. Wilson (*Plant Dis. Reptr*, 1962, **46,** 397—400).—Application of 100 p.p.m. of Agrimycin 100 (15% streptomycin SO_4 + 1·5% oxytetracycline) four times (2 bloom and 2 cover sprays) over 5 years to pear trees gave good control of fireblight. No antibiotic residues were found in or on pear fruit harvested 53 days after the last spraying and held for 64 days in cold storage. A. H. CORNFIELD.

Control of peach tree borer on young peach trees by a treatment before planting. E. H. Smith (*J. econ. Ent.*, 1962, **55,** 294—297).—Application of 50% Thiodan by dipping or painting the trunk while avoiding the root system gave complete control of *Sanninoidea exitiosa* on all trees. Of eggs placed on untreated trees 20% developed into larvae. In the laboratory, the rate of larval establishment on untreated wood was high. Dosages of 5 lb./100 gal. seemed near the threshold of complete effectiveness. C. M. HARDWICK.

Toxicity of some insecticides to larvae of codling moth after they enter apples. W. S. Hough (*J. econ. Ent.*, 1962, **55,** 378—381).—Apples were sprayed 1—6 days after the entry of *Carpocapsa pomonella* larvae and the emergence recorded. Malathion, parathion and phosphamidon were most effective for the first 3 days after entry and Zectran had the lowest effectiveness but the residues remained constant. The speed of penetration into the apple was very variable and phosphate insecticides and sevin slowed this considerably. C. M. HARDWICK.

Control of green peach aphid and pepper weevil on peppers. J. C. Elnore and R. D. Magor (*J. econ. Ent.*, 1962, **55,** 375—377).—Thiodan, Phosdrin and demeton were most effective in controlling *Anthonomus eugenii*. Demeton also controlled *Myzus persicae*. Parathion and malathion gave poor results. Phosphamidon and diazinon gave variable results. In general dusts were more effective than sprays. C. M. HARDWICK.

Peach tree borer experiments in peach orchards. O. I. Snapp (*J. econ. Ent.*, 1962, **55,** 418—419).—In experiments over several seasons trunk sprays of Thiodan, endrin and dieldrin gave greater reductions of *Sanninoidea exitiosa* than did parathion or Sevin. Ethylene dichloride emulsion applied to the tree base was effective. Trichlorobenzene was effective but phytotoxic. Soil application of phorate granules was ineffective. Sprays of *Bacillus thuriensis* were promising. C. M. HARDWICK.

Control of red scale, *Aonidiella aurantii*, on citrus. P. C. Hely (*Agric. Gaz. N.S.W.*, 1962, **73,** 70—72, 100—102).—The best control of red scale on citrus was obtained by two summer sprays of 2·5% white oil or white oil followed by 0·05% parathion. Oil-sprayed fruit had a better appearance than had fruit from non-sprayed trees and was freer of deep black-spot lesions. Post-bloom sprays of malathion or parathion increased the incidence of white wax scale. A. H. CORNFIELD.

Control of tomato yellow-top virus. B. M. Braithwaite and C. D. Blake (*Agric. Gaz. N.S.W.*, 1961, **72**, 627—629, 660).—Spraying field tomatoes with 0·025% Meta-isosystox every 7—10 days from seedling emergence to 3 weeks before the first fruit matured gave good control of aphids and tomato yellow-top virus. A. H. CORNFIELD.

Streptomycin resistance of the bacterial spot pathogen, *Xanthomonas vesicatoria*. R. E. Stall and P. I. Thayer (*Plant Dis. Reptr*, 1962, **46**, 389—392).—The proportion of streptomycin-resistant isolates of the bacterial spot pathogen increased after field sprays of streptomycin on tomato but not on pepper. Control of the disease was nullified by the increase in resistant bacteria. Isolates which differed in streptomycin resistance were all pathogenic. A streptomycin-susceptible isolate was controlled by streptomycin sprays in greenhouse tests with tomatoes, but a resistant isolate was not controlled. A. H. CORNFIELD.

Control of gummy stem blight or black rot, due to *Mycosphaerella melonis*, of cucurbits. Anon. (*Agric. Gaz. N.S.W.*, 1962, **73**, 24—26, 40).—Seed treatment with 0·1% $HgCl_2$ reduced the level of infection, but was not completely effective. Blight on foliage was controlled by application of zineb (1·5 lb. per 80 gal.). A. H. CORNFIELD.

Chemical control of slugs affecting vegetables and strawberries in the Pacific Northwest. A. J. Howitt and S. G. Cole (*J. econ. Ent.*, 1962, **55**, 320—325).—In several tests over 4 years the effectiveness of metaldehyde was most easily determined by establishing bait stations in plots. If rain followed a dust or spray treatment its effectiveness was reduced although all treatments reduced slug feeding. Metaldehyde suspension was unsatisfactory. The placing of metaldehyde dusts or sprays on the soil below the strawberry foliage greatly enhanced the effectiveness. (13 references.) C. M. HARDWICK.

Fusarium blight of soya-beans. J. Dunleavy (*Proc. Iowa Acad. Sci.*, 1961, **68**, 106—113).—*Fusarium orthoceras* produced necrosis of succulent root tissues of seedlings and infected tips of lateral roots of older plants. Pods and seeds were most susceptible to fungus penetration after maturity, and high R.H. was necessary for infection of seeds. Max. stand reduction and yield loss under field conditions were obtained when seeds were sown in *Fusarium*-contaminated soil before rains. In the greenhouse the disease was most destructive when plants were grown in soil at 100% water-holding capacity at 27°. (11 references.) E. G. BRICKELL.

Forecasting downy mildew of lima bean in Cape May County, New Jersey. R. A. Hyre, J. MacLeod and S. H. Davis, jun. (*Plant Dis. Reptr*, 1962, **46**, 393—395).—Forecasts of downy mildew, caused by *Phytophthora phaseoli*, on lima beans over 5 years were more accurate when based on rainfall and temp. data (*Plant Dis. Reptr*, 1959, Suppl. **257**, 179) or R.H. and temp. data (*Phytopathology*, 1960, **50**, 572) than when based on R.H. data (*Trans. Brit. mycol. Soc.*, 1947, **31**, 45). A. H. CORNFIELD.

Effect of certain fungicide treatments on tobacco mildew, in the field. A. Trouvelot and G. Stocky (*C. R. Acad. Agric. Fr.*, 1962, **42**, 481—488).—A description is given of the appearance of *Peronospora tabacina*, Adam, in 1958, the epidemic spreading rapidly in the tobacco fields of N.W. Europe in the wet summer of 1960. Of the fungicides tested, zineb and maneb (especially) applied as a powder rather than liquid spray gave the best results. E. M. J.

Effects of nitrogen, phosphorus and potassium on susceptibility of tobacco to *Alternaria* leafspot. J. S. Cole, L. F. Gates and R. C. Stephen (*Rhod. agric. J.*, 1961, **58**, 354—361).—High soil N delayed ripening of tobacco and increased *Alternaria* leafspot, particularly just before ripening. Responses to K were usually small, but K-deficient leaves were usually more susceptible and P-deficient leaves were particularly susceptible to leafspot. P applications hastened ripening and this may have accounted for their effects in reducing leafspot incidence. A. H. CORNFIELD.

Control of ratoon stunting disease of sugar cane. S. M. Lee and H. P. Liu (*Rep. Taiwan Sug. Exp. Sta.*, 1962 (27), 79—84).—Soaking diseased seed pieces for 2 h. at 50° in water was effective in controlling ratoon stunting disease. Germination of treated pieces was 95—97% and the average % of diseased cane was 1·83%. Hot-air treatment for 8 h. at 54° was not effective in eliminating the disease but in another year similar treatment at 55° was very effective. W. ELSTOW.

Control of Dutch elm disease. L. R. Schreiber and J. M. Harrison (*Plant Dis. Reptr*, 1962, **46**, 401—403).—Treatment of diseased trees with $ZnCl_2$ placed in holes bored in the trunk or with 'Tree Saver' (proprietary chemical placed in the trunk and on the soil around the tree) or with 'Soil Life' (proprietary org. soil amendment) did not affect the course of the disease. The two first named treatments injured the tree tissue. A. H. CORNFIELD.

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Evaluation of insecticides for control of smaller European elm bark beetle. C. C. Doane (*J. econ. Ent.*, 1962, **55**, 414—415).—In tests with DDT, AR/DDT [*NN*-di-*n*-butyl-*p*-chlorobenzenesulphonamide in DDT (1 : 5)] (**I**), heptachlor, methoxychlor, Thiodan (**II**) and Zectran, **I** and **II** were most toxic to *Scolytus multistriatus*. A high mortality within 24 h. was important in determining no. and size of feeding scores. C. M. HARDWICK.

Granular application of systemics for control of European pine shoot moth. H. M. Kulman and C. K. Dorsey (*J. econ. Ent.*, 1962, **55**, 304—305).—Spring applications of 0·6 oz. phorate to soil round small red pine trees controlled *Rhyacionia buoliana* in West Virginia for two seasons, starting in the first winter. Disyston acted similarly but had less residual effect. C. M. HARDWICK.

Growth and development of cotton as affected by toxaphene–DDT, methylparathion and calcium arsenate. L. C. Brown, G. W. Cathey and C. Lincoln (*J. econ. Ent.*, 1962, **55**, 298—301).—The effect of each insecticide on yield, boll production, no. of leaves, dry wt. of plant and its earliness is given. The timing of application was also significant. C. M. HARDWICK.

Imported fire ant toxic bait studies: GC-1283, a promising toxicant. C. S. Lofgren, C. E. Stringer and F. J. Bartlett (*J. econ. Ent.*, 1962, **55**, 405—407).—GC-1283 (dodecachloro-octahydro-1,3,4-methano-2*H*-cyclobuta[*c,d*]pentalene) is an analogue of Kepone. In toxicity tests it gave 100% as compared with 55% kill of *Solenopsis saevissima richteri* in 236 h. Baits containing GC-1283 did not repel the ants. In field tests it was slightly more effective than Kepone. C. M. HARDWICK.

Effect of acaricide–insecticide combinations on two-spotted spider mite and aphid populations on outdoor roses. T. J. Henneberry and E. A. Taylor (*J. econ. Ent.*, 1962, **55**, 332—334).—Weekly applications of Aramite, Tedion, Kelthane, Ovex and malathion or its combinations gave reduced no. of *Tetranychus telarius* while higher populations followed the use of DDT–lindane and methoxychlor. Sprays of DDT–lindane, methoxychlor–malathion or Thiodan reduced populations of *Macrosiphum rosae and M. euphorbiae* for 1 week. (14 references.) C. M. HARDWICK.

Effects of certain insecticides on earthworms. C. C. Doane (*J. econ. Ent.*, 1962, **55**, 416—418).—In plots treated with 2·8 lb. of DDT/acre earthworms acquired 21 p.p.m. DDT at 25 lb./acre was more toxic as a spray than as granules; counts made after 18 months showed that this dosage was very toxic. Chlordane and dieldrin at 10 lb./acre killed nearly all earthworms. Addition of lime had no significant effect. C. M. HARDWICK.

Effect of certain monovalent and divalent cations and detergents on plant virus infection. D. J. Rossouw (*Dissert. Abstr.*, 1962, **22**, 2936).—The effect of certain monovalent and divalent cations and detergents on the infectivity of six plant viruses (tobacco necrosis, cucumber mosaic, tobacco ringspot, southern bean mosaic, tobacco mosaic and turnip mosaic) was determined. Ba^{2+}, Ca^{2+} and Mg^{2+} were highly inhibitory to infectivity of four of the viruses. NH_4^+ was generally inhibitive to all the viruses. The effect of detergents upon infectivity was uncertain. F. C. SUTTON.

Recommended analytical methods for pesticides. VII. DDT Technical. Collaborative Pesticides analyt. Commee (CPAC) (*FAO Plant Protect. Bull.*, 1961, **9**, No. 8, 153—156, Reprint).—Great care is needed in sampling. For determining water and volatile products 5 g. are used. The org. Cl is converted into inorg. Cl with K ethylate which is then titrated with NH_4CNS. For the determination of 1,1,1-trichloro-2,2-di-(4-chlorophenyl)ethane (the chief component in DDT Technical), the sample is dissolved in aq. ethanol previously saturated with pure *pp'*-DDT at a selected temp. T° normally 25° ± 0·1°. The *pp'*-DDT is allowed to crystallise out at temp. T filtered and weighed. For the determination of acetone-insolubles, a 5-g. sample is refluxed with 150 ml. of acetone for 30 min., filtered and the residue weighed. E. M. J.

Analysis of cottonseed for residues of tributyl phosphorotrithioite. G. R. Boyd, jun. and M. A. Barber (*J. agric. Fd Chem.*, 1962, **10**, 196—198).—A method for determining residues of tributyl phosphorotrithioite, an effective defoliant, in cottonseed products is described. Na borohydride is used in the hydrolysis of the extracted trithioite to liberate butyl mercaptan, which is trapped in mercuric acetate solution and determined colorimetrically with *NN*-dimethyl-*p*-phenylenediamine. The method can detect 0·05 p.p.m. of the trithioite in a 100-g. sample. It cannot be applied to the analysis of crude cottonseed oil without a more efficient clean-up process. The hydrolytic technique should have application for similar S–P esters. W. ELSTOW.

Residues of Ethion on and in lemons and oranges as determined by an infra-red spectrophotometric procedure. F. A. Gunther, R. C. Blinn and G. E. Carman (*J. agric. Fd Chem.*, 1962, **10**, 224—226).—

Ethion (*OOO'O'*-tetraethyl *SS'*-methylenebisphosphorodithioate has insecticidal and acaricidal properties and shows promise for the control of red scale, *Aonidiella aurantii*, on citrus. By measuring the i.r. absorption at 1017 cm.$^{-1}$ and 959 cm.$^{-1}$ of residues stripped from the fruit and purified as described, a more specific estimation of Ethion residues is claimed than that by published colorimetric procedures. Results show that applied in the usual manner average residual half life for wettable powder formulations is 30 and 42 days for lemons and oranges respectively and from emulsifiable formulations 44 and 25 days respectively. W. ELSTOW.

Determination of total bromine residues in agricultural crops by instrumental neutron activation analysis. V. P. Guinn and J. C. Potter (*J. agric. Fd Chem.*, 1962, **10**, 232—236).—Neutron activation analysis is used to detect residues of bromide in crops treated with 1,2-dibromo-3-chloropropane. On irradiation ^{81}Br captures a neutron to give ^{82}Br with a half life of 36·0 h. which can be detected and identified by a NaI γ-spectrometer and its concn. measured by comparison with standards irradiated and counted under identical conditions. The method is rapid, is non-destructive and gives results agreeing well with chemical methods. In untreated crops Br levels were <10 p.p.m. but crops grown in fumigated soil had Br concn. of 20—200 p.p.m. (10 references.) W. ELSTOW.

Herbicides: their nature, mode of action and use. II. E. K. Woodford (*J. R. hort. Soc.*, 1962, **87**, 251—262).—Herbicides recommended for use on tree fruit, soft fruit, ornamentals and vegetables are tabulated. Their safe and efficient use is described. D. W. Robinson's long-term trials with blackcurrants and gooseberries indicate that cultivation of the soil may eventually be eliminated by the judicious use of herbicides. The use of specific herbicides is discussed. J. L. WALPOLE.

Control of weeds in pea crops. B. J. Hall (*Agric. Gaz. N.S.W.*, 1962, **73**, 15—17).—Although diuron (1—2 lb. per acre) as a pre-emergence spray did not give the best control of grass and broadleaf weeds, it gave the highest yields of peas. Atraton (2·5 lb.), Propazine (2·5 lb.) and prometryne (2·5 lb.) gave good control of grass and broadleaf weeds. A. H. CORNFIELD.

Control of herbaceous vegetation in forest plantings. E. W. Pruett and G. E. Gatherum (*Proc. Iowa Acad. Sci.*, 1961, **68**, 153—161).—In general, tree survival and height growth varied directly with the effectiveness of weed control as reflected in available soil moisture and light intensities. On cultivated plots response was greatest in the order of the following treatments: (i) Garlon and Esteron-Dowpon, (ii) simazine, Chloro IPC and mowing, and (iii) disking, vapam and Mylone. E. G. BRICKELL.

Control of capeweed, *Cryptostemma calendula*, L., Druce, in pastures. E. G. Cuthbertson (*Agric. Gaz. N.S.W.*, 1961, **72**, 630, 661—668).—Application of diquat (2—4 oz. active ingredient in 40 gal. per acre) to pastures in May, when capeweed was in the 3—5-leaf stage, gave good control of the weed. A. H. CORNFIELD.

Detection of traces of some triazine herbicides by gas–liquid chromatography. E. D. Chilwell and D. Hughes (*J. Sci. Fd Agric.*, 1962, **13**, 425—427).—Six triazine herbicides were separated by gas–liquid chromatography on glass ballotini columns with low loading of the liquid phase. A simple separation requires about 20 min. The method promises to be of use in determination of the persistence of triazines in the soil. It is less sensitive than the u.v. absorption method but has the advantage of speed. E. M. J.

Adducts of heterocyclic amides and thioamides with $\alpha\beta$-unsaturated compounds. Rohm & Haas Co. (B.P. 875,134—5, 3.12.57. U.S., 5.12.56).—Adducts, useful *inter alia* as fungicides (active against *Stemphylium sarcinaeforme*) are made by condensing in presence of strong alkali (NaOMe) the amide and [A] CH_2:CR·[CH_2]$_2$·CHMeR′, [B] CH_2:CR″R‴ (R and R′ are CN, substituted amide or an ester group; R″ is H or 1—8—C alkyl; R‴ is as R). Products made include [A] 1,3-*di*-(2,5-*dimethoxycarbonylhexyl*)-2-*oxoimidazolidine* and [B] 1-(2-*vinyloxyethyl*)-3-(2-*cyanoethyl*)-2-*oxoimidazolidine*, b.p. 166—168°/0·3 mm. H. S. R.

Spray oil compositions. Esso Research & Engng Co. (B.P. 873,317, 18.9.58. U.S., 28.4.58).—A composition for use in controlling fungus diseases on vegetation consists of a non-aq. mineral oil, η^{38} 65—88 sec. Saybolt, *d* 0·849—0·879, b.p. mainly 150—260°/10 mm., containing >15 wt.-% of aromatic and >2 wt.-% of olefinic hydrocarbons. F. R. BASFORD.

Heterocyclic thiophosphoric acid esters and pesticidal compositions containing them. ChemischeWerke Albert (B.P. 875,828, 30.12.59. Ger., 30.12.58).—Compounds of the general formula $(OR)_2PX{\cdot}S{\cdot}CH_2{\cdot}R'$ are claimed, also their use as active ingredients of pesticidal compositions (R is alkyl of 1—4 C; X is O or S; R′ is 2-oxo-2,3-dihydrobenzoxazol-3-yl radical optionally substituted in the benzo ring by halogen). In an example, the prep. is detailed of *Me*$_2$ S-2-*oxo*-2,3-*dihydrobenzoxazol*-3-*ylmethyl phosphorothiolothionate*, m.p. 68—72°. F. R. BASFORD.

***OO*-Dialkylphosphoric and thiophosphoric acid esters.** Montecatini Società Generale per l'Industria Mineraria e Chimica (B.P. 871,069, 2.10.57. It., 3.10.56. Addn. to B.P. 811,644; J.S.F.A. Abstr., 1960, ii, 95).—Compounds with insecticidal properties comprise esters of the general formula $(OR)_2PX{\cdot}OR'$ (R is alkyl of 1—4 C; X is O or S; R′ is coumarin-7-yl radical substituted in the 3,4-positions by a C chain which forms with the 3- and 4-C atoms a six-membered ring in which 1 of the C may be replaced by S if desired or when X is S, but when X is O then the six-membered ring may be saturated or unsaturated). Details are given for the prep. of *Et*$_2$ 3,4-*tetramethylenecoumarin*-7-*yl phosphate*, 59—60°. F. R. BASFORD.

Organic phosphorus compounds. CIBA Ltd. (B.P. 875,583, 21.10.57. Switz., 25.10.56).—Compounds useful as active ingredients of pesticidal compositions comprise org. P esters which may be represented by the formula $RX_m(R^IY_n){\cdot}PZ{\cdot}CR^{III}(COR^{IV}){\cdot}CO_2R^{II}$ or $RX_m(R^IY_n){\cdot}PZ{\cdot}O{\cdot}CR^{III}(:CR^{IV}){\cdot}CO_2R^{II}$ (*m* and *n* are 0—1; R—R^{II} are alkyl, cycloalkyl, alkenyl, aralkyl, aryl or heterocyclyl, optionally substituted, or R and R^I may form part of a ring system; R^{III} is H, alkyl or halogen; R^{IV} is alicyclic, aromatic or heterocyclic radical, but when R^{IV} is unsubstituted aryl and X, Y and Z are all O then R^{III} is alkyl or halogen, and when R^{III} is H or alkyl and X, Y and Z are all O, then there is not present an alkyl group containing at least 1 O-containing group apart from the OH group; X and Y are O, S, NH or NR; Z is O or S). In an example, Me_3PO_3 is treated with $\alpha\alpha$-dichloro-benzoylacetic acid in chlorobenzene at the b.p. to give a bright yellow oil. F. R. BASFORD.

Unsaturated alcohols and acyl derivatives thereof. F. Hoffmann-La Roche & Co. A.-G. (B.P. 874,581, 16.2.60. Switz., 16.2.59).—Compounds A·CH:CH·CH:CH·B (A and B are *n*-alkyl groups of 10—14 C, one of which contains terminal OH or acyl-O) are made by condensing A′·(C:C)$_m$·CHO with a phosphoran, viz., $Ph_3P^+{\cdot}\bar{C}H(C{:}C)_n{\cdot}B'$ (or the corresponding alkene) in an inert solvent (A′ and B′ are alkyl of 9—13 C, one of which contains a terminal CO_2H, esterified CO_2H, CH_2OH, or esterified or etherified CH_2OH; *m* is 1 and *n* is 0, or *vice versâ*), then, in any sequence, converting the triple bond into a double bond, and the terminal substituent into CH·OH or esterified CH_2·OH where necessary. A detailed example describes the prep. of *pentadeca*-6,8-*dienol*, b.p. 115—130°, in high vac. The products are insect attractants, and may be used in combination with insecticides. F. R. BASFORD.

Insecticidal compositions comprising a vinyl ester of a pentavalent phosphorus-containing acid and a synergist therefor. Shell International Research Maats. (B.P. 874,579, 30.11.59. U.S., 1.12.58).—There is claimed an insecticidal composition containing as active ingredient a compound of the general formula $X_2PY_2{\cdot}CR''{:}CR''{\cdot}COX$ (X is R, OR, NHR, NR_2, OR′R′·O, or O·R′·O·COR; Y is O or S; R″ is H, halogen or alkyl of 1—6 C: R is substituted or unsubstituted alkyl, aryl or aralkyl; R′ is alkylene, arylene or alkylene-arylene), e.g., malathion, and a synergist therefor, viz., a methylenedioxybenzene substituted in the 4-position and optionally in the 5-position by straight- or branched-chain alkyl or cycloalkyl optionally saturated (and containing at least one O, S, CO, CO_2 or SO group). A typical synergist is the acetal of acetaldehyde and 1 mol. respectively of 2-(2-ethoxyethoxy)ethanol and 3,4-methylenedioxyphenol. F. R. BASFORD.

Thiophosphoric acid esters. Farbenfabriken Bayer A.-G. (B.P. 874,570, 14.10.59. Ger., 18.10.58).—Compounds of the general formula $(OR)_2PO{\cdot}S{\cdot}CH_2{\cdot}S{\cdot}C_6H_4F$ are claimed (R is alkyl of 1—4 C); they have insecticidal properties and compositions containing them for use as such are also claimed. As an example the method of prep. is detailed of *Et*$_2$ p-*fluorophenylthiomethyl phosphorothiolate*, b.p. 109°/0·01 mm. F. R. BASFORD.

Insecticidal and acaricidal powders. Farbenfabriken Bayer A.-G. (B.P. 874,408, 15.9.59. Ger., 27.9.58).—The rain-resistance and wetting power of insecticidal and acaricidal spraying powders are increased by incorporation of a fatty alcohol (8—25 C) ether of a polyglycol (2—10) and a metal salt (1—5%) of a fatty acid of 8—25 C. The preferred salt is the Ca, Mg, Zn or Al salt of lauric, stearic, palmitic, margaric, oleic or linoleic acid. The ether may be stearyl alcohol polyglycol ether of 5—25 glycol residues or palmityl esterified with a polyglycol of 5—25 glycol residues. F. R. BASFORD.

Benzene hexachloride. Imperial Chemical Industries Ltd. (Inventors: F. R. Bradbury and H. M. Fox) (B.P. 875,877, 20.11.58).—A pesticidal pulverulent composition, useful as a dressing for seed, cereals and the like, comprises an admixture containing γ-benzene

hexachloride (**I**) and a solid chlorinated paraffin wax, wherein the ratio of **I** to wax is such that for v.p. measured by effusion at 50% v.p. of the **I** is <20% of that of free **I**, and after 6% has evaporated at 50° the v.p. is <8% of that of the free **I**. E. ENOS JONES.

Control of pests and the culture and protection of crops. Dow Chemical Co. (Inventor: C. R. Youngson) (B.P. 873,584, 22.5.59).—A composition suitable for use in the control of pests (especially soil-dwelling invertebrates which attack plant roots, e.g., nematodes and fungi) comprises an ethynyl ketone (e.g., butyn-3-one, pentyn-3-one or hexyn-3-one) and a carrier, viz., acetone, methylene dichloride, PhCl, petroleum distillate, surface-active agent, inert solid. F. R. BASFORD.

Herbicidal compounds and compositions. E. I. Du Pont de Nemours & Co. (B.P. 874,928, 18.12.59. U.S., 19.12.58, 11.3. and 14.4.59).—There is claimed a herbicidal composition containing a compound of the general formula NR;CX·NR′R″ (X is halogen; R and R′ are aliphatic radicals and R″ is aryl or is alkyl if R′ is aryl, the aryl group being Ph optionally substituted in other than one of the *o*-positions by 1—3 halogen, 1—2 alkyl of 1—4 C, or NO_2 or alkoxy of 1—4 C). The compounds are obtained by treating a substituted urea with P pentahalide at 10—140° in an inert solvent. NN-*dimethyl*-N′-*phenyl-chloroformamidine* has b.p. 96—99°/1 mm. F. R. BASFORD.

Compositions containing 3-amino-1,2,4-triazole for use in the economic eradication and/or suppression of undesirable plant growth. Amchem Products Inc. (B.P. 876,461, 1.2.60. U.S., 13.2.59).—A (synergistic) herbicidal composition, especially active against perennial grasses, e.g., quackgrass (*Agropyren repens* L. Beauv.) and Bermuda grass (*Cynodon dactylon* L. Pers.) contains 3-amino-1,2,4-triazole (0·5—2) and at least 1 alkali metal or NH_4 thiocyanate. F. R. BASFORD.

Organic complexes suitable for use as herbicides. E. I. Du Pont de Nemours & Co. (Inventor: R. S. Kittila) (B.P. 875,459, 6.11.59).—Complexes composed of equimol. of 2,3,6- or 2,3,5-trichloro-, or 2,3,5,6-tetrachloro-benzoic acid and a substituted urea, viz., NRR′·CO·NR″·R‴ (R is H or alkyl of 1—4 C; R′ is Me or Et; R″ is H or Me; R‴ is Ph with at least one free *o*-position and optionally substituted by 1—4 halogen or alkyl of 1—4 C) (e.g., 3-*p*-chlorophenyl-1,1-dimethylurea) are claimed as herbicides. F. R. BASFORD.

Animal Husbandry

Effect of particle size on the *in vitro* cellulose digestibility of forages by rumen bacteria. B. A. Dehority and R. R. Johnson (*J. Dairy Sci.*, 1961, **44**, 2242—2249).—Mature timothy hay was ground in a ball mill for varying periods. Cellulose digestibility, in *in vitro* tests, increased with the period of milling, the relative increase being greater with the stage of maturity and degree of lignification of the material. Of the residual (undigested) cellulose <5% was digested in a second test, but when the residue was again milled 67% became digestible. The amount of cellulose sol. in cupriethylenediamine declined with advancing maturity of the hay. Results obtained support the view that lignin in forage acts as a barrier to cellulose. A. G. POLLARD.

Chemical and biological evaluation of protein feeds for poultry. I. Ascarelli and B. Gestetner (*J. Sci. Fd Agric.*, 1962, **13**, 402—410).—As indexes of protein quality the following methods were tried: (*a*) for fish meals Bender's Net Protein Utilisation (NPU) method for rats was adapted to chickens (Leghorn cockerels). A new regression of water/N was calculated. Loss of available lysine by high-temp. processing of the fish meal was confirmed; no correlation between hydroxyproline content and nutritive value as assessed by the NPU method was found. (*b*) With soya-bean meal all degrees of heating were differentiated by Cresol red and good correlation with biological results was shown. Methods based on measurement of antitryptic or urease activity showed satisfactory correlation with the nutritive value of under- or sufficiently heated meals. Heating the meal for 15 min. at 120° doubled the amount of available methionine. Available lysine decreased in the laboratory-prepared meals according to the length of the heat treatment. (*c*) With cottonseed meals nutritional differences between commercial samples could not be found by tryptic digest examination or determination of available lysine. Biological tests with cottonseed meal, soya-bean meal and mixtures of the two showed the advantage of the use of mixed rations in comparison with that of cottonseed meal only. (43 references.) E. M. J.

Micronutrients in soils and plants in relation to animal nutrition. J. F. Hodgson, R. M. Leach, jun. and W. H. Allaway (*J. agric. Fd Chem.*, 1962, **10**, 171—174).—Although forage plants may be growing well without any sign of a deficiency of micronutrients, e.g., Cu, Zn, Fe, Mn, Co, Se, I, Mo or B, they may still not provide enough of certain of these trace elements for the animals feeding on them. This is particularly the case with Co. The micronutrient content and requirements of plants and animals are discussed with a view to controlling such deficiencies. (33 references.) E. ELSTOW.

Relation between chemical composition and nutritive value of Uganda grasses. R. M. Bredon and B. Marshall (*Nature, Lond.*, 1962, **194**, 702—703).—The relationship found between chemical composition and nutritive values for various Uganda grasses has been expressed in the form of three regression equations. With these, it should only be necessary to determine crude protein, fat and total ash to obtain the full nutritional value of other grasses. (12 references.) S. A. BROOKS.

Measurement of the nutritive value of lucerne and timothy hay by varied techniques. J. G. Archibald, H. Fenner, D. F. Owen, jun. and H. D. Barnes (*J. Dairy Sci.*, 1961, **44**, 2232—2241).—Digestibility values were determined by use of rumen-fistulated cows either by insertion of the chopped forage in Dacron bags into the rumen or by the conventional examination of the faeces over a 10-day period. The former technique indicated the digestibility of the dry matter, fibre, ether extract, energy, cellulose, lignin and pentosans of timothy to be equal or to exceed the corresponding values for lucerne; lucerne gave higher values for protein and N-free extract. The latter method yielded similar results except that the value for lignin was higher in the case of lucerne. The amounts of volatile fatty acids and NH_3 in the rumen were higher when lucerne or high-protein timothy was fed than when medium-protein timothy was used. A. G. POLLARD.

Nutritive evaluation of forages for ruminants: animal performance, nutrient digestibility and volatile fatty acid production. R. A. Alexander (*Dissert. Abstr.*, 1962, **22**, 2925).—In four groups of experiments the nutritive value of forages grown under various management practices was studied. On an average, cattle digested all nutrients, except crude fibre, more efficiently than did sheep. The variation among individual animals of a given species was greater for sheep than for cattle. Regression equations are presented for calculating digestion coeff. from one to the other species. F. C. SUTTON.

Detection of antibiotics in fodder. R. Louis (*Mitt. Lebensm. Hyg., Bern*, 1961, **52**, 575—580).—For qual. detection of Aureomycin and Terramycin the paper chromatographic method of Selzer and Wright having a stationary phase of a citric acid–phosphate buffer of pH 3·5 and a mobile phase of a mixture of $CHCl_3$–nitromethane–pyridine (10 : 20 : 3) is recommended. When the flow boundary is about 10 cm. from the start point the chromatogram is dried in warm air. Both tetracyclines appear as yellow fluorescent spots, more clearly defined on treatment with NH_3. For quantities <10 mg./kg. the dried chromatogram (whole or strips) are laid on an inoculated agar plate. After incubation, inhibition of bacterial growths is shown by Aureomycin and Terramycin. For the quant. determination the agar plate method (Loch plate) is used. After incubation the dia. of the inhibition zones and the activity of the antibiotic substance are measured. Turbidimetric methods (described) have the advantage of a short incubation period (3—4 h.). E. M. J.

Determination of iodine in mineral premixes [for livestock feeds]. C. King, E. S. Bretz and T. J. Kneip (*Analyt. Chem.*, 1962, **34**, 565—567).—The sample containing >5 mg. of I is digested with $KClO_3$ and HCl to convert I^- to IO_3^- and the evolved Cl_2 is absorbed in aq. NaOH (forming OCl^-). Formic acid is added to the digestion flask and I liberated is distilled over into the aq. NaOH solution to reform IO_3^- which is determined colorimetrically. For premixes containing thymol iodide (as distinct from Ca iodate or ethylenediamine dihydriodide) preliminary treatment with K oxalate for 10 h. at 500° is necessary. T. R. ANDREW.

Thyroxine turnover methods for determining thyroid secretion rates in dairy cattle. T. B. Post and J. P. Mixner (*J. Dairy Sci.*, 1961, **44**, 2265—2277).—Of methods examined preference is given to an isotope dilution technique in which diminution in the specific activity of protein-bound I following injection of ^{131}I-labelled L-thyroxine is measured. From data thus obtained with bull calves and with non-lactating cows, the average thyroid secretion rates were 0·40 and 1·14 mg./100 lb. live wt. respectively. A. G. POLLARD.

Natural herbage of the subtropics. I. Digestibility of herbage grazed by cattle. II. Effect of added concentrates on digestibility of herbage. J. H. Topps (*J. agric. Sci.*, 1962, **58**, 387—391, 393—397).—I. The herbage consumed by cattle has a higher digestibility of org. matter than of that collected, this effect increasing as the season advances. This indicates that cattle become more selective in their grazing. Marked yearly differences in digestibility–faecal N relationships and in the quality of foliage occur.

II. The digestibility of the org. matter of all mixed feeds is higher

than that of the corresponding herbage without added concentrates, a mixture of early summer herbage, 4, and dairy grazing meal, 1 part, giving the highest increase. M. LONG.

Production of valuable substances in biological purification of industrial sewage. U. Behrens and K. Sattler (*Mikrobiologiya*, 1962, **31,** 344—349).—Treatment of sewage containing also phenols and org. acids from brown coal low-temp. carbonisation and coking plants is discussed, and a scheme for biological purification of such waters to give valuable active sludge is described. The dry sludge produced contained 40% of protein, in which 17 amino-acids were identified. One g. of dried sludge contained 0·15 mg. of vitamin B_{12}, Cyanocobalamin amounted to ⅓ of B_{12}, another ⅓ was factor B and remainder was factors $B_{12\,777}$ (*sic*), A, pseudo vitamin B_{12} and unidentified factors. Active sludge used as an additive (1—5%) to the feed of rats, birds and pigs, increased wt. gain by 10—40%, without harm to the animals. P. W. B. HARRISON.

Influence of weight of sample on the precision of determination of vitamin A in animal foods. R. Ferrando and P. Mainguy (*Ann. Falsif., Paris*, 1962, **55,** 26—33).—Determinations of vitamin A on 60 samples (5 g.) of barley flour containing a commercial vitamin A concentrate show a wide spread of results due to the large particle size (0·25 mm.) of vitamin A concentrate. It is advisable to take 100-g. samples and to make duplicate determinations.
J. V. RUSSO.

Rôle of pregastric esterase in the abomasal hydrolysis of milk fat in the young calf. H. A. Ramsey and J. W. Young (*J. Dairy Sci.*, 1961, **44,** 2227—2231).—The production of free fatty acids in the abomasum was examined in calves fed orally and by fistula (to minimise the amount of pregastric esterase entering the abomasum). Yields of total free fatty acids and also their separation into butyric, valeric and higher acids are discussed in relation to the probable part played by the enzyme in the digestion of milk fat by calves.
A. G. POLLARD.

Comparative nitrogen digestibility in Brahman, Brahman × Shorthorn, Africander × Hereford and Hereford steers. G. C. Ashton (*J. agric. Sci.*, 1962, **58,** 333—342).—Steers with Zebu blood are superior to Herefords with regard to apparent digestibility of dry matter and N, although the differences disappear when N digestibility is calculated on the basis of (feed N — undigested faecal residue N)/feed N. Differences in dialysable faecal N appear between breed groups, the amount being highly negatively correlated with body wt.
M. LONG.

Utilisation of Hyparrhenia veld for the nutrition of cattle in the dry season. III. Digestibility of the produce of mature veld and veld hay, and the effect of feeding supplementary protein and urea. C. A. Smith (*J. agric. Sci.*, 1962, **58,** 173—178).—Mature Hyparrhenia veld (**I**), harvested at the end of the rains, leads to protein deficiency. The nutritive value of mature **I**, as standing hay, falls progressively and voluntary intake falls simultaneously. Since dry matter intake falls, a mid-season mature herbage diet is greatly deficient in energy and protein. The intake of **I** is increased by supplementary protein or urea. The main factor limiting the utilisation of **I** is a dietary protein deficiency. M. LONG.

Effects of dietary sodium fluoride on dairy cows. VI. In young heifers. J. W. Suttie, R. Gesteland and P. H. Phillips (*J. Dairy Sci.*, 1961, **44,** 2250—2258).—Calves were given various levels of NaF (F, 1—2 mg./kg. body wt., daily). Observations of F retention in bones, urinary F, gain in wt., adult wt., tooth scores, systemic physiological effects and general health showed the tolerance level to be <1·6 mg. of F/kg. body-wt./daily. The toxic threshold was circa 1·4 mg. daily and was unchanged when the age at initial dosage varied from 6 weeks to 6 months; it was not influenced by ingestion of F by the dam. The best index of F toxicity was the occurrence of bone-F >5500 p.p.m. A. G. POLLARD.

Comparison between pelleted and chopped lucerne hay in the feeding of lambs. F. J. Van Der Merwe, I. L. Ferreira, L. P. Vosloo and D. G. F. Labuschagne (*S. Afr. J. agric. Sci.*, 1962, **5,** 109—121).—Compared with chopped hay, ground and pelleted hay markedly improved the digestibility, feed intake and growth response in the case of a poor quality hay when fed *ad lib.* Average figures for pellet fed lambs were 0·42 lb. per day gain in live wt. and a conversion ratio of 1 : 8·52 compared with 0·12 lb. and 1 : 18·46 for lambs on chopped hay. With good quality hay there was no significant difference between the response of groups of animals fed pellets and those fed chopped hay due to the animals selecting and consuming a higher digestible energy fraction of the chopped hay; animals fed individually on pellets gained significantly faster than those fed in groups. W. ELSTOW.

Energy requirements of sheep for maintenance and gain. I. Pen fed sheep. I. E. Coop. **II. Grazing sheep.** I. E. Coop and M. K. Hill (*J. agric. Sci.*, 1962, **58,** 179—186, 187—199).—I. The estimate for the maintenance of a 100-lb. sheep, using the ¾ power of live-wt. concept, is digestible org. matter 0·92 lb., TDN 0·96 or S.E. 0·89 lb.
II. Three trials indicate that grazing sheep require higher values of digestible organic matter intake per day than pen sheep—1·36 to 1·63 lb. This extra requirement is due to energy costs of walking and harvesting, as well as climatic factors. M. LONG.

Supplementary vitamin D_3 for lambs. S. Curran and J. P. Crowley (*Irish J. agric. Res.*, 1961, **1,** 43—48).—Parenteral administration of vitamin D_3 to lambs within 21 days of birth did not affect growth rates up to 50—70 days. Indications were obtained of an increased rate of growth of twins following treatment during the first few days after birth. A. G. POLLARD.

Experimental production of hypomagnesaemia in ewes and its control by small magnesium supplements. N. S. Ritchie, R. G. Hemingway, J. S. S. Inglis and R. M. Peacock (*J. agric. Sci.*, 1962, **58,** 399—404).—On low-Mg diets, consisting of barley straw, sucrose and casein, a daily 0·25 g. MgO drench maintains the plasma-Mg at a higher level than that given by unsupplemented diets. Experimental Mg heavy pellets have the same effect. The unsupplemented diet induces hypomagnesaemia. M. LONG.

Early weaning of pigs. VIII. Copper sulphate as growth stimulant. I. A. M. Lucas, R. M. Livingstone, A. W. Boyne and I. McDonald (*J. agric. Sci.*, 1962, **58,** 201—208).—$CuSO_4$ 0·1% in the diet has no ill effect on piglets and improves gain and feed conversion efficiency even when introduced immediately after weaning. A supplementary antibiotic has further slight beneficial effects. No further benefit is found after 55 lb. wt. M. LONG.

Isoleucine requirement of the weanling pig. R. E. Evans (*J. agric. Sci.*, 1962, **58,** 413—422).—No benefit from barley-rich diets is obtained by increasing the isoleucine content from 0·59 to 0·74%. An improvement is, however, shown by increasing the isoleucine content from 0·55 to 0·68% for pigs on a maize diet. Growth is then almost as good as with pigs receiving 10% of white fishmeal.
M. LONG.

Effect of addition of copper sulphate to the feed of growing pigs on the live weight increase, the feed utilisation and copper content of individual tissue. H. Jucker (*Mitt. Lebensm. Hyg., Bern*, 1961, **52,** 580—588).—Basal diets for pigs, with and without animal protein, were supplemented with $CuSO_4$ (0—375 p.p.m.). Wt. gains in pigs of about 20 kg. increased 3—12%, up to 100 kg. live wt. Feed conversion was improved 3—7%. With increase in $CuSO_4$ in the feed Cu was significantly and progressively accumulated in the liver, the Cu level in fresh tissue rising from 16 p.p.m. in unsupplemented pigs to 312 p.p.m. (15 references.) E. M. J.

Effects of oxytetracycline and copper sulphate, separately and together, in the rations of growing pigs. R. Braude, J. M. Townsend, G. Harrington and J. G. Rowell (*J. agric. Sci.*, 1962, **58,** 251—256).—Growth rate and efficiency of food conversion are both improved by the addition of $CuSO_4$ (**I**) (2 lb./ton), and of oxytetracycline (**II**) (10 g./ton). **I** is more effective than **II**, but the effects are not additive. The cold dead wt. of the pig is increased by the supplements, carcasses being shorter and streaks thicker. **II** increases fat over eye muscle and **I** increases depth of eye muscle. Percentage yields of fore-end, middle and gammon are unaffected.
M. LONG.

Amino-acid supplementation of diets rich in maize meal for weaning pigs. R. E. Evans (*J. agric. Sci.*, 1962, **58,** 209—218).—A soya-bean diet (**I**) plus 0·3% of L-lysine hydrochloride and 0·2% of DL-methionine is as satisfactory as a fishmeal diet. As little as 10% digestible crude protein supports max. growth when the essential amino-acids are in balance. Groundnut meal does not replace **I** satisfactorily.
M. LONG.

Protein-energy relationships in the diet of the chick. J. B. O'Neil, J. Biely, G. C. Hodgson, J. R. Aitken and A. R. Robblee (*Poultry Sci.*, 1962, **41,** 739—745).—The effect of dietary protein (16—28%) together with varying productive energy (750—950 kcal. per lb.) on performance of chicks to 4 weeks of age was studied at five locations. An excess of productive energy in relation to the protein level of the diet depressed growth rate and feed efficiency. An excess of protein in relation to productive energy did not adversely affect growth or feed efficiency, but was wasteful of protein. A. H. CORNFIELD.

Net protein values [NPV] for the growing chicken from carcass analysis with special reference to animal protein sources. J. D. Summers and H. Fisher (*J. Sci. Fd Agric.*, 1962, **13,** 496—500).—The carcass N-retention procedure developed by Bender and Miller was adapted for use with chickens. NPV results are reported, e.g., for casein (**I**), gelatin (**II**), whole egg protein (**III**), egg albumin (**IV**), Menhaden fish meal (**V**) and meat meal (**VI**). **III** and **IV** were the best protein sources tested, but NPV results were lower than those reported for rats. Crude casein was improved by supplementation with arginine, glycine and methionine, but the NPV of the supple-

mented protein was not as good as that of isolated soya-bean protein with added methionine (**VII**). Zein and **II** gave NPV of approx. 20; the birds lost wt. **II** supplemented with essential amino-acids to simulate **VII** gave NPV of approx. 40 compared with 67 for **VII**; **V** gave 63 and **VI** 36. The high **II** or collagen content of **VI** is responsible for low NPV. (14 references.) E. M. J.

Utilisation of non-protein nitrogen by the chick. W. R. Featherston (*Dissert. Abstr.*, 1962, **22**, 2964—2965).—Natural, low protein diets were used, and essential amino-acids were added, to meet the requirements of the chick. Ground yellow maize and soya-bean oil meal were the principal constituents of the basal diets. A positive correlation was observed between the levels of amino-acids in the diet and those in the plasma. Significant positive correlations were also observed between the levels of total, essential and non-essential amino-acids in the plasma and the rates of growth of chicks. F. C. Sutton.

Utilisation of hydrolysed feather meal by chicks. I. R. Sibbald, S. J. Slinger and W. F. Pepper (*Poultry Sci.*, 1962, **41**, 844—849).—Feather meal had a metabolisable energy of 1 kcal. per g., or approx. 19% of its gross energy content, indicating that the material was poorly utilised by the chick. In low-protein diets feather meal was inferior to both soya-bean oil meal and meat meal, but in rations containing adequate amounts of the essential amino-acids up to 6% of the soya-bean oil meal could be replaced by feather meal without ill effects. The nutritive value of feather meal was due only partly to its Zn content. A. H. Cornfield.

Serum and egg cholesterol levels in mature hens as influenced by dietary protein and fat changes. H. M. Edwards, jun., J. E. Marion and J. C. Driggers (*Poultry Sci.*, 1962, **41**, 713—717).—Variations in dietary protein (17—22%), kcal./protein ratio (69—93) and the addition of 10% of various fats to the hen's diet had little effect on cholesterol % in the egg fat. Maize oil (10%) in the hen's diet increased the I val. of the egg fat, whilst beef tallow and lard had no effect. A. H. Cornfield.

Methionine supplementation of the diet of laying hens. R. H. Harms, C. R. Douglas and P. W. Waldroup (*Poultry Sci.*, 1962, **41**, 805—812).—Methionine was a limiting factor in a maize–soya-bean oil-meal type diets for laying hens. Supplementing the diets with 0·075% methionine hydroxy analogue calcium (MHA) resulted in improved performance in two out of three tests. The energy content of the diet was a factor involved in controlling the response to MHA supplementation. A. H. Cornfield.

Growth response of chicks to antibiotics from 1950 to 1961. D. A Heth and H. R. Bird (*Poultry Sci.*, 1962, **41**, 755—760).—The average wt. of chicks fed procaine penicillin (0·004—0·030 g. per kg. of feed) were 108·5% and 108·8% of the wt. of basal groups during the periods 1950—1953 and 1956—1960 respectively. With the tetracyclines (0·010—0·035 g. per kg. of feed) the wt. were 112·3% and and 110·2% respectively for the two periods. For the period 1956—1959 Zn bacitracin produced an average wt. of 105·9% of the basal when supplied at 0·010—0·035 g. per kg. and 115·2% of the basal when supplied at 0·100 g. per kg. of feed. A. H. Cornfield.

Calcium and phosphorus requirements of growing turkeys and chickens. S. D. Formica, M. J. Smidt, M. M. Bacharach, W. F. Davin and J. C. Fritz (*Poultry Sci.*, 1962, **41**, 771—776).—Broiler-strain chicks required ≮0·6% each of Ca and P for satisfactory growth to 4 weeks of age. Broad Breasted Bronze turkey poults grew normally to 8 weeks on a diet containing Ca 0·81 and P 0·65% and to 24 weeks of age on a diet containing Ca 0·83 and P 0·56%. Higher levels of these elements, either alone or in combination, did not improve performance further. A. H. Cornfield.

Action of antibiotics in stimulating the growth of poultry. I. Effect of feeding lysed *Escherichia coli* and faecal preparations. W. K. Warden and P. J. Schaible (*Poultry Sci.*, 1962, **41**, 725—732).—Addition of 0·1% of lysed and lyophylised contents of *Escherichia coli* cells, still retaining enzyme activity but having no antibiotic activity, stimulated growth of chicks to the same extent as did antibiotics. Addition of 1%, but not lower levels, of spray-dried lysed *E. coli*, to a P-deficient diet produced normal growth and improved bone development in the same way as did $CaHPO_4$. Administration of a saline faecal solution by crop inoculation depressed wt. gains. Addition of lysed cells to a diet containing Zn bacitracin or penicillin did not stimulate wt. gains further. Oxytetracycline, which was ineffective alone, stimulated growth when added to a diet containing lysed cells. Mortality was high only in the absence of added P or lysed cells. Antibiotics increased feed efficiency, lysed cells had no effect, and faecal prep. reduced feed efficiency. A. H. Cornfield.

Antibacterial agents. Beecham Research Laboratories Ltd. (B.P. 874,414—6, 11.5.60. U.S., 25.5.59).—Antibacterial agents, suitable for use in animal feeds (and also as nutritional supplements), for the treatment of mastitis in cattle and of infectious diseases in poultry and animals, comprise penicillanic acids substituted in the 6-position by [A] $NH{\cdot}CO{\cdot}[CH_2]_n{\cdot}R$, [B] $NH{\cdot}CO{\cdot}C_6H_4{\cdot}CO_2H$-*o*, [C] $NH{\cdot}CO{\cdot}[CH_2]_2{\cdot}C_6H_3R'R''R'''$ (*n* is 2—4, R is cyclohexyl and R'—R''' are various substituents). Examples are [A] 6-(*δ-cyclohexylvaleramido*)-, [B] 6-(*o-carboxybenzamido*)- and [C] 6-(*β-phenylpropionamido*)-*penicillanic acid*. Non-toxic salts are also claimed. H. S. R.

Penicillin derivatives. Beecham Research Laboratories Ltd. (Inventors: F. P. Doyle, J. H. C. Nayler and H. Smith) (B.P. 873,049, 6.10.58 and 12.5.59).—Penicillin deriv., useful as antibacterial agents, animal-feed supplements, agents for the treatment of mastitis, etc., are obtained by acylation of 6-aminopenicillanic acid with $CO_2H{\cdot}CH(NH_2){\cdot}[CH_2]_n{\cdot}R$ or a salt thereof, in which the NH_2 group is protected (the protecting group being subsequently removed) (*n* is 0 or an integer; R is H, NH_2, CO_2H or substituted or unsubstituted alkyl, aryl, aralkyl or heterocyclyl). In an example, *carbobenzoxyaminomethyl penicillin* is prepared and its Na salt is subjected to hydrogenolysis in presence of $Pd/BaCO_3$ to give the *Na salt* of *aminomethyl penicillin*. F. R. Basford.

Polyene alcohols and acyl derivatives of same. F. Hoffmann-La Roche & Co., A.-G. (Inventors: O. Isler, R. Ruegg and U. Schwieter) (B.P. 875,761, 28.8.58).—Aldehydes $R{\cdot}CH{:}CH{\cdot}CMe{:}CH{\cdot}CH{:}CH{\cdot}CMe{:}CH{\cdot}C{\vdots}C{\cdot}CH{\vdots}CMe{\cdot}[CH{:}CH{\cdot}CH{:}CMe]_m{\cdot}[CH{:}CH]_n{\cdot}CHO$ or compounds obtained therefrom by converting the acetylenic into an ethylenic bond (R is 2,6,6-trimethylcyclohexenyl; *m* is 0—3; *n* is 0—1) are converted with alkali metal hydride into the corresponding alcohols. Acyl derivatives of the latter are also claimed. The compounds are useful as colour additives for foodstuffs (either directly or indirectly, e.g., in the feeding of poultry) or as intermediates in the synthesis of other carotenoids. In an example, the prep. is detailed of 17-(2,6,6-*trimethylcyclohexenyl*)-2,6,11,15-*tetramethylheptadeca*-2,4,6,8,10,12,14,16-*octaen*-1-*ol* m.p. 148—149° (*acetate*, m.p. 130—132°). F. R. Basford.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Grain storage studies. XXXII. Quantitative changes occurring in the sugars of wheat deteriorating in the presence and absence of moulds. B. T. Lynch, R. L. Glass and W. F. Geddes (*Cereal Chem.*, 1962, **39**, 256—262).—Sound wheat stored for 8 weeks at 30° and 20% moisture in air, N_2 or CO_2 all showed extensive deteriorative changes. Under aerobic conditions most changes were due to mould growth, but in anaerobic conditions, although fat acidity was stable, there were extensive changes in sugar content (decrease of sucrose, increase of glucose, fructose and galactose), germination was reduced to zero and the samples gave flour of extremely poor baking quality with very sticky doughs. (13 references.) E. C. Apling.

Rice quality factors. V. Determination of a differentiated outer layer in cooked rice. VI. Influence of protein on cooking quality. Protein in the outer layer. E. Primo, A. Casas, S. Barber and C. Benedito de Barber (*Rev. Agroquím. Technol. Aliment.*, 1962, **2**, 130—134, 135—141).—V. Cooked rice shows a thin and discontinuous outer layer covering the translucent interior of the grain. This outer layer is readily separated by erosion on stirring in water followed by screening and drying, for which standard conditions are described. Wide differences in the proportion of outer layer are found between varieties, but these show little relation to cooking quality.

VI. Determination of the protein content of the outer layers of different rice varieties shows that the higher the protein content the less the tendency of the cooked grains to cohere. Due to heterogeneous N distribution in the endosperm, the protein content of the outer layers appears to be a better index of cooking quality than the protein content of the whole grain. (16 references.) E. C. Apling.

Detection of rice flour in wheat flour. J. Seidemann (*Dtsch. LebensmittRdsch.*, 1962, **58**, 168—169).—Observations of shape, size, staining reactions, appearance in polarised light, or rate of solution in chloral hydrate show insufficiently distinct differences between rice starch and the small starch grains of wheat for the certain detection of additions of rice starch to wheat flour, and differences of gelatinisation temp. show a clearer distinction. (52·5 to 65·0° for

wheat starch ; 67·5 to 78·0° for rice starch.) If the sample is heated in water at 65 to 67°, wheat starch is gelatinised (swollen) and ungelatinised rice starch granules are readily recognised microscopically by their polyhedral, sharp-angled and three- to six-sided shape. E. C. APLING.

Refining of millet flours. I. Ragi *(Eleusine coracana).* P. P. Kurien and H. S. R. Desikachar (*Food Sci., Mysore*, 1962, **11**, 136—137).—A method of removing husk from ragi, without excessive loss of nutrients, by dry milling followed by wet milling is described. M. O'LEARY.

Carotenoids of maize and sorghum. I. Analytical procedure. C. W. Blessin (*Cereal Chem.*, 1962, **39**, 236—242).—The simplified method described depends on extraction of total pigments with ethanol, separation of carotenes and xanthophylls by column chromatography on Celite (9 : 1 n-hexane/acetone for elution of carotenes, 3 : 1 n-hexane/ethanol for xanthophylls) and their spectrophotometric determination at 451 and 445 mμ respectively). (13 references.) E. C. APLING.

Evaluation of colour characteristics of flours obtained from various types and varieties of wheat. T. Yasunaga and M. Uemura (*Cereal Chem.*, 1962, **39**, 171—183).—Flour brightness as evaluated by the Kent-Jones and Martin Colour Grader, or the brightness method of Cereal Laboratory Methods, depends on the amount of bran contamination and the colour characteristics of the bran. A study of the spectral reflectance curves and extractable pigments of flour and bran from various sources is reported and a ' colour index ', K_B is proposed for evaluating flour colour irrespective of bran contamination. K_B = [log (reflectance at 554 mμ) − log (reflectance at 700 mμ] − 0·021/log (reflectance at 554 mμ) − 0·0119. The proposed method makes possible the evaluation of the commercial value of wheat samples from the viewpoint of good flour colour from reflectance measurements made at two wavelengths on model mill flour. E. C. APLING.

Influence of late nitrogenous fertilisation on the protein composition of cereals. L. Völker (*Getreide u. Mehl*, 1962, **12**, 33—36).—If late N fertilisation leads to an increase in crude protein then there is also a change in amino-acid distribution in the protein. The lysine fraction decreases and the glutamic acid fraction increases with increasing crude protein content. (15 references.) J. V. RUSSO.

Ratio of protein to moist gluten content. P. F. Pelshenke and H. Bolling (*Getreide u. Mehl.*, 1962, **12**, 29—33).—A linear relationship between protein and moist gluten is found ; % protein = a + b × % moist gluten. Values of a and b vary with the year of harvest over the period 1959—1961. J. V. RUSSO.

Amino-acid composition and changes in wheat with regard to quality and storage damage. P. Linko (*Getreide u. Mehl*, 1962, **12**, 25—29).—The effects of temp. time and R.H. of storage on free amino-acid contents of wheat are reviewed. Typical paper electrophoresis patterns are obtained for sound and damaged wheat. Glutamic acid decarboxylase activity determined by a Sandstedt–Blish manometer is a useful routine quality indicator. (27 references.) J. V. RUSSO.

Radiochemical method for estimation of sulphydryl-to-disulphide ratio in wheat gluten. C. C. Lee and E, R. Samuels (*Canad. J. Chem.*, 1962, **40**, 1040—1042).—After reaction of ^{35}S-labelled gluten with *N*-ethylmaleimide and hydrolysis of the product with 6N-HCl, the ^{35}S-labelled *S*-(*N*-ethylsuccinimido)-L-cysteine (**I**) is isolated with the aid of ordinary **I** (*idem, ibid.*, 1961, **39**, 1152) as carrier. Separation of **I** from cystine and methionine is effected by two-dimensional ascending chromatography of the conc. hydrolysate with the solvent mixtures 1-butanol–pyridine–acetic acid–water (for ~18 h.) and water-saturated phenol (for ~20 h.) ; spots are revealed with ninhydrin and the activity of the spot corresponding to **I** is counted. Recovery of **I** is ~80% always ; results for reduced glutathione in water agree with the known contents. W. J. BAKER.

Binding of protein to various phosphorus-containing compounds in gluten. A. Bourdet and J. Herard (*Getreide u. Mehl*, 1962, **12**, 61—70).—A progress report on the fractionation of lipids from 10 flour samples and their separated glutens in relation to baking quality. The ratios of bound/free lipid were much higher in gluten (2—3 : 1) than in flour (0·12—0·24 : 1). Phospholipids form an almost constant proportion of the free lipids of flour but from 35 to 50% of the bound lipids of gluten. A fixation constant (bound lipids of the gluten/free lipids of the flour), depending on the propensity of the flour protein to bind lipids, varied from 3·5 to 5·4 and may possibly be related to baking quality. In gluten, P occurs as mineral orthophosphate (14 to 27% of total P) and in org. combinations of lipid, phytin and nucleic acid nature. Lipid P in the bound lipids (inseparable from protein except by hydrolysis) accounts for 16 to 22% of total P and includes simple lipids based on choline, and other lipids containing also galactose and arabinose. Phytin P accounts for 30 to 46% of total P and the nucleic acid fraction includes deoxyribonucleic acid (11 to 18% of total P) and ribonucleic acid (3 to 17% of total P). (29 references.) E. C. APLING.

Effect of iodate and *N*-ethylmaleimide on extensigraph properties of flour. W. Bushuk and I. Hlynka (*Cereal Chem.*, 1962, **39**, 189—195).—*N*-Ethylmaleimide (**I**), an -SH blocking agent, and iodate both show normal improver effect when used at comparable concn. in doughs mixed for 2½ min. under N_2 in the GRL mixer. When added at a rate higher than the accessible -SH content of the dough, ' reverse improver effect ' is shown by **I** at mixing times >5 min. This reverse effect is also shown by iodate at mixing times >15 min. in the absence, but not in the presence, of NaCl. The reversal appears to be due to dough breakdown by the mixer action, which is partially inhibited by salt, and is particularly rapid in -SH blocked doughs. At the longer mixing times dough breakdown is more rapid in the presence of atm. O_2. E. C. APLING.

Flour protein solubility and baking quality. III. Continuous progressive extraction. E. Maes (*Getreide u. Mehl*, 1962, **16**, 70—72).—Sol. proteins are continuously extracted and simultaneously fractionated from flour (10 g.) intimately mixed with fine pumice powder (10 g.) and fine sand (100 g.) and filled into a glass column (25 cm. × 3·5 cm. dia.) between 1·5 cm. layers of sand and quartz. The column is percolated successively with water (200 ml.), 40% isopropanol (150 ml.), 3·85% lactic acid (Zeleny reagent) (150 ml.), 0·5% KOH (150 ml.) and water (70 ml.). The method has very good reproducibility. E. C. APLING.

Plant lipids. IV. Glycerides and phosphatides in cereal grains. F. Aylward and A. J. Showler (*J. Sci. Fd Agric.*, 1962, **13**, 492—496).—The lipids of barley, oats and rye were extracted with ether and ethanol ; glycerides and phosphatides were separated by pptn. with acetone and the phosphatide fraction separated again into ethanol-sol. (lecithin) and -insol. portions. By gas chromatography the main fatty acids in the three cereal lipids were shown to be palmitic, oleic, linoleic and linolenic acids, palmitic being the predominant saturated acid and linoleic (5—6%) the most widely distributed unsaturated acid, followed by oleic which is found in large quantities in oats. The phosphatides differ from the glycerides of the same cereal in their content of palmitic and oleic acids ; in general the lecithins show more unsaturation and the cephalins more saturation than the corresponding glycerides. E. M. J.

Rapid method for the extraction of lipids from wheat products. C. C. Tsen, I. Levi and I. Hlynka (*Cereal Chem.*, 1962, **39**, 195—203).—The rapid method of Folch *et al.* (*J. biol. Chem.*, 1957, **226**, 497—509) is adapted to the extraction of lipids from wheat products, including baked materials. The sample (20 g.) is homogenised in a Waring Blendor for 2 min. with a mixture of ethanol (50 ml.), $CHCl_3$ (25 ml.) and water (sufficient to make 20 ml., including the moisture natural to the sample), then for 30 sec. after further addition of $CHCl_3$ (25 ml.) and for 30 sec. after addition of water (25 ml.). The homogenate is centrifuged at 0° for 10 min. at 10,000 × g, the $CHCl_3$ layer separated and an aliquot is filtered, washed, evaporated and dried at 50° in vac. and weighed. The method gives results comparable with those obtained by extraction with ethanol or water-saturated n-butanol, but gives higher values than extractions with light petroleum. (17 references.) E. C. APLING.

Quantitative rapid fat determination method for flours and flour mixtures. E. Mohr and K. Franke (*Brot u. Gebäck*, 1962, **16**, 58—60).—The use of a butyrometer for determining fat in flour and flour products is described. The sample is first dispersed in water (for self-raising powder N/10-H_2SO_4), 10 ml. conc. H_2SO_4, an aliquot of the dispersion and 1 ml. amyl alcohol and sufficient water to bring the total water to 12 ml. are placed in the butyrometer. The contents are well mixed and the butyrometer is centrifuged for 5 min. in a heated centrifuge and is kept for a further 5 min. in a water bath at 70°. The fat vol. is read and the fat content calculated. The results are in good agreement with the standard HCl hydrolysis method. J. V. RUSSO.

Further observations of flour lipids ; their participation in oxidative reactions in doughmaking. J. B. M. Coppock and N. W. R. Daniels (*Brot u. Gebäck*, 1962, **16**, 117—119).—Gas chromatographic examination of the methyl ester–fatty acid components of the lipids extracted from flour, dough and bread showed the presence of unidentified acids X and Y, not present in untreated flour, in the lipids from doughs, but not in the lipids of the resulting bread. Amounts of X and Y were increased by increasing treatment with KIO_3. The suspected rôle of O_2 in the formation of X and Y was confirmed by finding that no X and Y were formed in untreated doughs prepared in a laboratory ' Do-Maker ' (in which mixing takes place in a closed reservoir), only small amounts were found in KIO_3-treated ' Do-Maker ' doughs, and that in KIO_3-treated doughs mixed in air,

amounts of X and Y were highest near the surface of the dough. Higher amounts of X and Y were also found in doughs from ClO_2-treated flour than in doughs from untreated flour, and a third unidentified acid, Z, was detected in dough prepared from ClO_2-treated flour with the addition of 1% of linoleic acid.
E. C. APLING.

Determination of thiamine in wheat flour. J. R. Fraser and R. Sawyer (*Analyst*, 1962, **87**, 230).—The Food Standards Committee 'Report on Bread and Flour' [H.M.S.O., London, 1960] recommends use of the 'direct' method of Ridyard (*Analyst*, 1949, **74**, 18) for determination of thiamine in flour. In this method results are corrected for 'non-thiochrome fluorescence'. In later work by Ridyard (*ibid.*, 1961, **86**, 723) it is suggested that no blank correction is necessary. A comparison of results by both methods now confirms that the blank correction is unnecessary. Results of further work now proceeding on a microbiological assay with *Lactobacillus viridescens* show only a slight tendency to be higher than those by the direct method.
A. O. JONES.

Breakage of endosperm cell walls in flour milling. W. E. Schulze and M. M. MacMasters (*Cereal Chem.*, 1962, **39**, 204—209).—Microscopical study of hard red winter wheat flour shows that cell-wall particles from the starchy endosperm are always portions of walls of two adjacent cells with the cementing middle lamina, showing that cell-wall breakage is invariably transverse. (16 references.)
E. C. APLING.

Experiences in the determination of starch damage. E. C. Apling, D. W. Kent-Jones and A. J. Amos (*J. Sci. Fd Agric.*, 1962, **13**, 516—520).—Because the amount of damaged starch in a flour affects water absorption and the course of panary fermentation, frequent determinations of starch damage are necessary. In milling or baking laboratories the procedure of Greer and Stewart in conjunction with the maltose test of Blish and Sandstedt can be applied as a routine procedure. In 10 samples of flour each examined in two laboratories (**I**) and (**II**), the results from **I** were slightly higher than those from **II**. Other data show the greater susceptibility to damage of the starch of hard wheats during the milling process; the mean damaged starch content of Manitoban flours was 6·2% and that of soft flours 4·5%.
E. M. J.

Present state of knowledge on the action of amylases in the manufacture of bread, biscuits and cakes. R. Drapron (*Brot u. Gebäck*, 1962, **16**, 108—116).—A general review discussing the physical and chemical structure of starch; general properties of amylases and their modes of action on amylose and amylopectin; the rôle of the amylase in doughmaking, panary fermentation and the baking of bread, cakes and biscuits; differences in the activity and thermal stability of malt, bacterial and fungal amylases; amylase supplementation and control of excess amylolytic activity in flour; and future perspectives. (29 references.)
E. C. APLING.

Rheological properties of flour dough. Shear and compression. J. Glucklich and L. Shelef (*Cereal Chem.*, 1962, **39**, 242—255).—Studies in the rheological behaviour of durum doughs are reported and the rheological parameters of durum and vulgare doughs are compared. Doughs from both types of wheat exhibited similar rheological behaviour. At ordinary stresses durum dough was more rigid and more viscous than vulgare dough, but at higher stresses its rigidity became equal to that of a vulgare dough. (13 references.)
E. C. APLING.

New acid dough problems. F. Dworak (*Brot u. Gebäck*, 1962, **16**, 41—45).—Optimum conditions for the prep. of acid doughs (rye, wheat and mixed) by the one-stage process are tabulated. The concn. of yeast bacteria at the start of the process is of importance. In mixed breads more succulent loaves with better keeping properties are obtained if a dark rye flour is combined with a light wheat flour.
J. V. RUSSO.

Standardisation of baking methods. A. Schilerud (*Brot u. Gebäck*, 1962, **16**, 53—55).—In 1960 samples of flours were sent to 18 laboratories in 16 countries for baking tests. Wide variations in results were obtained due to widely varying techniques. Standardisation of dough temp., resting time, dimensions of baking tins, oven temp. and baking time is relatively simple. It is more difficult to agree on an optimum water absorption and on dough handling conditions.
J. V. RUSSO.

Rôle of lipids in oxidation of doughs. C. C. Tsen and I. Hlynka (*Cereal Chem.*, 1962, **39**, 209—219).—Examination of extracted flour lipids by the thiobarbituric acid method shows that lipid peroxides are formed in dough during mixing in air or O_2. Lipoxidase (commercial 'Wytase') increased peroxidation, but NDGA and propyl gallate reduced peroxidation to close to the level of doughs mixed in N_2. Added peroxides increased the structural relaxation constant of dough, suggesting a similar improving rôle of the peroxides of flour lipids. -SH blocking agents, e.g. *p*-chloromercuric benzoate (PCMB), iodoacetic acid (IAA), *N*-ethylmaleimide (NEMI) and $HgCl_2$, and oxidising improving agents, increased peroxidation, suggesting competition between -SH groups and flour lipids for O_2 in dough and that peroxides strengthen dough by oxidising -SH groups. (29 references.)
E. C. APLING.

Determination of polyoxyethylene glycol in bakery products. P. A. Veitch and J. B. Jones (*Cereal Chem.*, 1962, **39**, 220—224).—Polyoxyethylene(8)monostearate is separated from bread by extraction of acid-hydrolysed bread crumbs with $CHCl_3$, absorption on a column of alumina, elution with ethanol and is semi-quant. determined by ascending paper chromatography on 3 MM Whatman paper, using a mixture of butanol (200 ml.), aq. NH_3 (1 + 10) (100 ml.) and ethanol (48 ml.) as developing solvent and a modified Dragendorf reagent [bismuth subnitrate (850 mg.) dissolved in 20% acetic acid (50 ml.), mixed with KI (8 g.) in water (20 ml.), filtered, kept in the dark and diluted 1 : 12 with 20% acetic acid before use] as chromogenic agent. The method overcomes the false positive results obtained on bread containing lecithin-based emulsifiers by Garrison's colorimetric method (*J. Ass. off. agric. Chem., Wash.*, 1957, **40**, 1085).
E. C. APLING.

Two polypeptide antibiotics elaborated by *Saccharomyces cerevisiae*. R. J. Robinson, B. S. Miller, J. A. Johnson, B. Curnutte and T. H. Lord (*Cereal Chem.*, 1962, **39**, 183—188).—Two antibiotic substances (**I** and **II**) isolated from *S. cerevisiae* ATCC 9896 strain 139 during fermentation were purified and shown to be polypeptides. **I** contained glutamic acid, serine, glycine, alanine, valine, leucine and tryptophan. **II** contained the foregoing and γ-aminobutyric acid, aspartic acid and phenylalanine. The inhibitory actions of the antibiotics towards *Staphylococcus aureus* were not destroyed by baking.
E. C. APLING.

Ammonium bicarbonate as a raising ingredient for baked goods. W. Sturm and E. Hanssen (*Dtsch. LebensmittRdsch.*, 1962, **58**, 164—168).—The chemical and physical properties of snythetically prepared NH_4HCO_3 are reviewed and its suitability as a raising agent for baked goods, due to its high CO_2 content, lack of non-volatile residue, and ready solubility, is argued. Its main disadvantage is its tendency to clump on storage if exposed to moisture. Residual NH_3 found in a large no. of baked goods raised with NH_4HCO_3 varied from 2 to 170 mg. per 100 g., without consistent relation to thickness or moisture content of the goods. NH_3 contents similar in amount were also found in a variety of bakery raw materials (e.g., egg albumin, cocoa, milk powder, etc.), and it is emphasised that these residuals are sufficient to seriously interfere with 'crude protein' determinations by the Kjeldahl method unless corrections for free NH_3 are made. (26 references.)
E. C. APLING.

Souring in bread on extended storage. E. Drews (*Brot u. Gebäck*, 1962, **16**, 101—108).—'Crust-free' mixed rye-bread, wholemeal bread and pumpernickel were packed in foil and in boxes and stored for up to 240 days. In all cases acidities (total titratable, lactic, and lactic + acetic) showed a min. at 56 to 70 days and no appreciable storage souring occurred.
E. C. APLING.

Pasteurisation of liquid whole egg and evaluation of the baking properties of frozen whole egg. A. J. Amos, C. L. Heller, B. C. Hobbs, B. C. Roberts and M. E. Smith (*J. Hyg., Camb.*, 1962, **60**, 135—143).—Adequate pasteurisation of the whole egg can be attained at 64·4° for a minimum holding time of 2·5 min. Large-scale baking trials and subsequent trade acceptance, showed that this treatment was in all ways satisfactory.
C. V.

Determination of creatinine in products containing carbohydrates. H. J. Hardon and H. A. Kok (*Mitt. Lebensm. Hyg., Bern*, 1962, **53**, 1—5).—The Hadorn method gives results which are too high when applied to the determination of creatinine in soups (vegetarian or otherwise); passage through Al_2O_3 followed by extraction with Et_2O fails to remove all interfering impurities. Satisfactory results can be obtained by substituting the extraction with Et_2O by passage through Amberlite IR-120[H] on which the remaining impurities are adsorbed. The purified creatinine is eluted with 1% aq. NH_3, and then determined as usual. (12 references.)
P. S. ARUP.

[A] **Temperature profiles of thickening agents in high-temperature short-time [HTST] and retort processing.** [B] **Effect of high-temperature short-time processing on the viscosity and colour of five thickening agents.** A. S. Kiratsous, F. J. Francis and J. W. Zahradnik (*Food Technol.*, 1962, **16**, No. 7, 107—111, 111—114).—[A] The effects of HTST (280°F) and conventional (240°F) processing systems on temp. profiles and sterilisation values of five different types of thickening agents were compared. The following were used: tap water, sucrose 10% in water, non-fat milk and whole milk to which were added maize starch, 2 and 4%, waxy maize starch, 2 and 4%, gum tragacanth 0·5 and 0·75%, carboxymethylcellulose 0·2 and 0·4% and Na alginate 0·25 and 0·5%. Extensive data are given, e.g., sterilisation values of heating and cooling profiles. After the temp. profiles were obtained the F_o values could be calculated

assuming a 'z' value of 18. F_0 values for 40 formulations are presented. Heating and cooling phases combined, contributed ⩽50% and as much as 66% of the total F_0 values. A HTST process computed on the basis of F_0 during holding times only, can reduce the holding time by 50% at $F_0 = 10$ and still obtain a commercially sterile product. (18 references.)

[B] The maize starch and the waxy maize formulations showed a decrease in η of respectively 73 and 42% from that of the unprocessed gelatinised controls when processed by the HTST method. The retort process gave a corresponding decrease of 50 and 23%. Gum tragacanth (**I**), carboxymethylcellulose (**II**) and Na alginate (**III**) respectively, gave decreases of 32, 17 and 54 for the HTST method, and 93, 86 and 91 for the retort process. **III** formulations with milk showed some protein coagulation in the retort samples. **I** and **II** formulations with milk showed protein coagulation with both processes. Colour of starch formulations in water and sucrose was quite different for the two methods, with a higher brightness factor in the HTST samples. The colour differences for the other thickeners were quite small, while for the milk series they were large for all formulations because of the effect on the milk itself.

E. M. J.

Flour milling process. Jyun-Ichi Goto (B.P. 876,372, 19.12.57. Jap., 24.12.56).—There is claimed an improved process for milling flour (especially wheat flour) by means of a relatively low friction and low pressure, whereby destruction of the cell is minimised. Apparatus is figured. F. R. Basford.

Shortenings. Unilever Ltd. (B.P. 874,182, 5.2.58. U.S., 11.2.57.)—A shortening composition for use in baking and frying (to give, e.g., high-volume cakes with tender crusts, even close grain, and soft texture) consists of edible liquid oil (cottonseed oil) and 1—8% of a stearin comprising polyacid triglycerides, viz., $(C_3H_5O_3)RR'R''$ (R is acyl radical of a saturated fatty acid of 20—26; R' and R'' are acyl groups of fatty acids of 16—26 C, at least one of these groups being different from R, and ⩽25 mol.-% of the stearin being triacid triglycerides, I val. ⩾20, in which R, R' and R'' are different). The preferred stearin is a hydrogenated stearin obtained by solvent winterisation of groundnut oil, and the composition should have $\eta^{21} \geqslant 5000$ cP. F. R. Basford.

Bread. T. Barker & Sons Ltd., G. L. Coxon and J. H. Parry (B.P. 875,218, 8.5.59).—A bakery moulding machine (for use in the production of stratified or sectional bread without recurrent changes in crumb texture and colour) is figured and claimed.

F. R. Basford.

Sugars and confectionery

Effect of various sugars on browning. Y. Pomeranz, J. A. Johnson and J. A. Shellenberger (*J. Fd Sci.*, 1962, **27**, 350—354).—The rate of browning of 20 sugars or sugar derivatives was followed by reflectance measurements of cookies and spectrophotometric measurements of browning of dilute buffered solutions of the sugars and glycine or lysine heated at 114° in an autoclave. The order of decreasing reactivity for the pentoses was: ribose, xylose and arabinose; of the hexoses, galactose was the most reactive and rhamnose the least. Raising the pH into the alkaline range enhanced browning. Lysine was more active in inducing browning than was glycine. (11 references.) E. M. J.

Gas production as a result of γ-irradiation of sugar syrups. D. Emery, P. Burchill, D. Hamerski and G. Germann (*Food Technol.*, 1962, **16**, No. 7, 124—125).—γ-Irradiation of aq. sugar syrups within an enclosed system results in a substantial internal pressure. This has important implications in the consideration of the radiation preservation of high sugar food items in gas-impermeable containers. E. M. J.

Specificity of commercial preparations of glucose oxidase. R. S. Crowne and K. R. L. Mansford (*Analyst*, 1962, **87**, 294—296).—The specificity was investigated of several commercial preparations of glucose oxidase classified as 'pure' (activity ≃11,000 units per μg.) and 'crude' (1100 units per μg.) towards pure maltose, maltotriose and maltotetraose separated chromatographically from liquid glucose. The solution of the sugar being studied (50—100 mg. per ml.) is incubated at 37° with a mixture of the powdered commercial glucose oxidase, commercial horse radish peroxidase and *o*-dianisidine made up to 500 ml. with a phosphate buffer (pH 7). The H_2O_2 formed by action of the glucose oxidase on the sugar is catalysed by the peroxidase to oxidise *o*-dianisidine and the resulting chromogen is measured spectrophotometrically at 450 mμ. Results show that both the crude and pure glucose oxidase preparations available commercially have pronounced glycosidal activity, and that even the pure preparations are not completely specific for glucose.

A. O. Jones.

Changes occurring in bee honey during large-scale drawing-off. H. Hadorn and K. Zürcher (*Mitt. Lebensm. Hyg., Bern*, 1962, **53**, 28—34).—Losses of 35—50% of the initial diastase and saccharase activities and increases of 80—114% in the hydroxymethylfurfural (**I**) content are observed in honey after it has been melted in 300-kg. containers for 5 days at 48°. After melting at 43° for 5 days, the losses in enzymic activity are small, whilst the gains in **I** are 30—40% of the initial value. The change in the content of **I** is more sensitive to heat than the change in diastatic activity. A suitable max. limit for **I** would be 3 mg. per 100 g. P. S. Arup.

Rheometry of chocolates. II. Measurement of flow thresholds and their calculation by the Casson equation. W. Heimann and A. Fincke (*Z. Lebensmittl Untersuch.*, 1962, **117**, 225—230).—In continuation of previous work (cf. *ibid.*, 93), measurement of the min. shearing stress required to produce permanent deformation in the material are made in a viscometer in which the rotating cylinder has been grooved in order to avoid slipping. After temp.-equalisation of the sample with the apparatus at 37·8°, the cylinder is set in motion at 0·0314 rev. per sec. during 5 min. (exactly) and then suddenly stopped. The residual tension in the driving-spring is a measure of the flow threshold. At residual tensions >100 dynes/sq. cm. the values obtained for molten chocolate agree within 10% with those calculated by the Casson formula; at lower tensions the agreement is less satisfactory. For molten milk chocolate and cacao masses the experimental values are generally much greater than the calculated values. (See col. 36 below.)

P. S. Arup.

Preparing a gel using a carboxymethyl dextran. Commonwealth Engng Co. (B.P. 876,927, 10.12.57).—The gel is prepared by incorporating in water at pH 3·0—7·0, 0·2—1·0% by wt. of a carboxymethyl ether of a readily water-sol., high-mol.-wt. dextran containing an average of 0·8—1·5 carboxymethyl groups per anhydroglucose unit, prepared by interaction of the dextran with chloroacetic acid in presence of NaOH. E. Enos Jones.

Fermentation and Alcoholic Beverages

Determination of iron in wine. Examination of rapid methods for suitability and usefulness in practice. H. Hadorn (*Mitt. Lebensm. Hyg., Bern*, 1962, **53**, 35—42).—Preference is given to the Rentschler and Tanner method (cf. *Schweiz. Z. f. Obst-u. Weinbau*, 1961, **70**, 514) which is based on the addition of a series of known amounts of $K_4Fe(CN)_6$ to the sample, boiling and filtering, and the estimation of the min. amount of $K_4Fe(CN)_6$ required for the pptn. of all the Fe by tests on the filtrates. The bipyridyl method gives accurate results, but is not suitable for routine testing. The Lambert ferrometer method is not sufficiently accurate. P. S. Arup.

Presence of α-methylmalic acid in wines. —. Dimotaki-Kourakou (*Ann. Falsif., Paris*, 1962, **55**, 149—158).—The presence of this acid is demonstrated by paper-chromatographic tests. The α-methylmalic acid (**I**) can, like citric acid (**II**), be determined by the Kogan-Penaud method viz., by oxidation with $KMnO_4$ at pH 3·2—3·4 to $COMe_2$ which is determined by iodimetric titration. The separation of **I** and **II** is accomplished by fractional elution of the acids of wine from a column of Dowex 1X2 (acetate form) with 2·5N-NaOH. The **I** can be determined (after the elimination of the volatile acids) in the fraction containing malic and other acids; the **II** is determined in the fraction containing the **I** and tartaric acid. It is shown that the increase in acidity in wine during fermentation is due, not to an increase in **II**, but solely to the continuous production of **I** (max. 80—103 mg./l.), the extent of which is proportional to sugar content of the wine. The formation of **I** by a pure culture of yeast in sterilised must is demonstrated. These facts discount the view that **I** is formed by the bacterial decarboxylation of **II**. The determination of **I** is accurate within 3% and that of **II** within 8%. (16 references.)

P. S. Arup.

Spectrophotometric determination of sorbic acid in wines. A. Maurel and —. Touye (*C. R. Acad. Agric. Fr.*, 1962, **48**, 268—272).—In order to obtain a quant. yield of the acid by steam-distillation it is necessary to collect 300 ml. of distillate from 20 ml. of wine (acidified with tartaric acid). The λ of max. absorption of sorbic acid in aq. solution varies appreciably with the pH. Determinations can conveniently be made at pH 8—12, as the max. at 254 mμ remains constant in this range. P. S. Arup.

Flavour spectrum of apple-wine volatiles. J. S. Matthews, H. Sugisawa and D. R. MacGregor (*J. Fd Sci.*, 1962, **27**, 355—362).—Sixteen compounds in the volatile fraction of apple wine were isolated and identified. As extraction solvent ethyl chloride was preferred against pentane or isopentane, because it could be removed from the extract at room temp. and it gave higher yields of low-

boiling compounds. The volatiles were separated and purified by gas–liquid chromatography. The compounds were identified by comparing retention times, i.r. spectra and m.p. of deriv. with those of known compounds. (11 references.) E. M. J.

Kilning of very light and typically dark malt. A. Kaiser (*Brauwissenschaft*, 1962, **15**, 175—185).—In this, the fifth, section of a detailed discussion of classical and modern kilning systems, practical examples of the kilning of dark malt are described in detail. Exact techniques are given and results quoted for the same green malt kilned in both horizontal and high-velocity kilns. From these and the effects of malting procedure, information is obtained on the formation of melanoid compounds. Finally, reference is made to the construction and purpose of two-level kilns built 30 years ago. J. B. Woof.

Mechanism of action of malt β-glucanases. II. Separation and characterisation of malt endo-β-glucanases. W. W. Luchsinger (*Cereal Chem.*, 1962, **39**, 225—235).—Fractionation of an extract of barley green malt with $(NH_4)_2SO_4$ yielded three fractions containing endo-β-glucanase activity. The optimum pH was 4·75 for fraction i and 4·55 for fractions ii and iii. Fraction ii was inactivated by dilution with distilled water, but was stabilised by reduced glutathione, cysteine, barley gum or NaCl. The determined heat stabilities of the fractions showed half-inactivation times of 0·7 h. at 40° for i and 5·2 h. for iii and stabilised ii. (10 references.) E. C. Apling.

Aeration of wort and its inoculation. R. Scriban (*Brasserie*, 1962, **17**, 125—133).—Theoretical and practical considerations bearing on the dissolution of atm. O_2 in wort and on the importance of adequate aeration are explained. Installations in general use for the transport of wort from the coolers to the vats are critically examined from this point of view. Greatly improved results can be obtained by injecting compressed air through aeration cylinders (or 'candles') made of porous sintered metal (Poral Inox. C) situated so as to meet the current of cooled and filtered wort in a state of turbulence. Three suitable installations are described. The yeast is injected at the point of max. turbulence, viz. near the top of the vertically placed cylinder. (29 references.) P. S. Arup.

[A] Effects of aeration on continuous fermentations operating at high yeast concentrations. [B] Acceleration of continuous fermentations by yeast autolysates. G. Harris and N. R. Merritt (*J. Inst. Brew.*, 1962, **68**, [N.S. **59**] 241—244, 244—246).—[A] In closed system continuous fermentation in the production of beer, in which the yeast is retained in the fermenter, restriction of air lowers the rate of beer production. The steady state of efficiency established when air is allowed to diffuse into the fermenter, is lowered when access of air is prevented and lowered slightly further when the ingoing wort is deoxygenated, but rises again on aeration or diffusion of air. However, the max. loss of efficiency on restriction of air in this closed system was only 20% against 80% in the system in which the yeast escapes freely from the fermenter with the beer. The uptake of N by the yeast is also reduced when aeration is reduced.

[B] By adding autolysates of the yeast emerging from the fermenter in continuous fermentation processes, to the ingoing wort, considerable increase in efficiency of beer production is obtained. both yeast growth and fermentation rate being increased. The process is considered to be simpler than that of recycling the yeast. The amino-acid composition of the autolysate is similar to that of brewer's whole yeast except for the complete absence of arginine. (13 references.) J. I. M. Jones.

Effect of gibberellic acid on the growth of hops. J. B. Roberts and R. Stevens (*J. Inst. Brew.*, 1962, **68** [N.S. **59**], 247—250).—Large-scale field trials were carried out in 1960 and 1961 in which hops, variety Bullion, were sprayed with 12·5 p.p.m. of the K salt of gibberellic acid at the time of burr formation. Samples of treated and untreated material were collected at intervals and examined for total resin, α-acids and essential oil (oxygenated and hydrocarbon fractions). Fifteen days after spraying, the treated hops were considerably more developed, the wt. of 100 cones of each being respectively 4·3 and 1·4 g. and the resin and acid content considerably higher in the treated hops. By harvest time, however, analytical differences had disappeared, but the yield from the treated areas was only 80—90% of that from the untreated areas. (13 references.) J. I. M. Jones.

Problem of gushing. A. Ferdinandus, J. Gombert and H. E. Jansen (*J. Inst. Brew.*, 1962, **68**, [N.S. **59**] 250—253).—Beer of poor attenuation and hence of high N content was bottled and submitted to sea transport for some weeks. Some of the crown corks on the bottles had Al linings and others PVC linings; some bottles were packed vertically and some horizontally. After 8 to 12 weeks they were examined for 'gushing' and the released gases analysed by gas chromatography. Gushing occurred when initial N was high and when dissolved H_2 was high. High H_2 values occurred only in bottles with Al-lined crown corks stored horizontally. In a second experiment part of a high-N beer was washed with CO_2 before bottling. Reduction of the N thus produced abolished gushing. (10 references.) J. I. M. Jones.

Determination of anthocyanogens. II. Further studies on the analysis of beer. W. D. McFarlane and M. J. Vader (*J. Inst. Brew.*, 1962, **68** [N.S. **59**], 254—257).—Agent AT (a polymer of *N*-vinylpyrrolidone) and nylon were compared as adsorbents for the determination of anthocyanogens in beer. Both compounds were found to contain impurities that reduced the yield of anthocyanidin pigments from the anthocyanogens. This was prevented by purifying the adsorbents or, alternatively, by adding ascorbic acid along with the adsorbent. Purification of AT was effected by boiling in 10% HCl then washing; that of nylon by dissolving in boiling 20% HCl and re-pptg. with cold water, washing and drying. The brown coloured compounds produced from impurities in the beer in the test are adsorbed by nylon but not by AT. The greater selectivity of the latter for beer anthocyanogens therefore makes the colorimetric test based on its use more sensitive and accurate and the quantity required is about one-fifth that of nylon. J. I. M. Jones.

Particle size of beer turbidigens and its influence on nephelometry. R. S. W. Thorne and K. Svendsen (*J. Inst. Brew.*, 1962, **68** [N.S. **59**], 257—270).—The relations between abs. turbidity (measured spectrophotometrically), nephelometric turbidity (measured at 90° to the incident light) and particle size in beers were studied. Abs. turbidities were measured at 410 and 610 mμ or at 550 mμ. An empirical relation between abs. turbidity at 550 mμ and those at 410 and 610 mμ is given by $T_{550} = (T_{410} + T_{610})T_{610}/T_{410}$. The ratio of $T_{610} : T_{410}$ (R) is adopted as an index of particle dia. Though R is not a linear function of the dia., over the greater part of its range in 75 beers studied, it increases with increasing dia. In these samples, R ranged from 0·09 to 1·43 and its standard error calculated from replicates was ±0·009. It is estimated that this range covers a range of particle dia. of 0·2 to 0·4 μ. Calibration of a nephelometer and a spectrophotometer with aq. formazin suspensions does not enable valid deductions to be made for beers. The ratio of nephelometric turbidity to absolute turbidity at 550 mμ was 0·54 for formazin suspensions but for the 75 beers tested it ranged from 0·30 to 1·25. The theoretical effect of particle size on nephelometry is discussed and the errors of nephelometry are reviewed. (11 references.) J. I. M. Jones.

Brewers' wort. A.P.V. Co., Ltd. (Inventor: G. A. Dummett) (B.P. 876,113, 30.5.57. Divided out of B.P. 848,641).—In the processing of brewers' wort in a continuous method in which the wort is in continuous movement from a mashing stage to a fermentation stage, the wort is passed at, e.g., 220°F, to a vessel or vessels maintained at an internal pressure such as to cause flashing-off of certain constituents (and effect simultaneous purification and concentration of the wort). F. R. Basford.

Fruits, Vegetables, etc.

Rapid estimation of moisture in dried apples. S. M. Sykes and G. G. Coote (*Commonw. sci. industr. Res. Org. Aust., Div. Fd Pres.*, 1962, tech. Paper No. 29. 11 pp.).—An electrical conductance moisture meter developed for the Californian Dried Fruits Association was used satisfactorily in processing factories in Tasmania. Values of moisture content in the 16—26% range could be predicted with a standard error of ±0·8% for a single reading on one sample. A modified instrument known as the 'Stowell' meter (described) reduced the standard error to one half of the above value under comparable conditions. In a survey of bulk heaps in factories, heap to heap and factory to factory differences were highly significant. The need for the mixing of the product in each heap and for allowing sufficient time for equilibration to obtain a uniform moisture content is emphasised. E. M. J.

Concentration of volatiles in controlled atmosphere storage of apples and their relation to storage operations. J. Johansson (*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 137—145).—The levels of C_2H_4 and non-ethylenic volatiles in the atm. in 'controlled atm.' apple storage rooms are presented. Water scrubbing reduced the concn. of both fractions, whilst the use of activated C reduced the concn. of only the non-ethylenic volatiles. A. H. Cornfield.

Factors influencing susceptibility of pears to carbon dioxide injury. E. Hansen and W. M. Mellenthin (*Proc. Amer. Soc. hort. Sci.*, 1962, **80**, 146—153).—Development of CO_2 injury (brown-core) in pears stored at −1·1° in plastic-lined boxes was not related to specific CO_2

concn., but varied according to the degree of susceptibility of the fruit to injury. Incidence of brown-core was high when fruit was picked late in the season, with unfavourable handling practices (delayed storage, slow cooling rates), with fruit from trees of low vigour, and when O_2 concn. in the storage atm. was reduced greatly. A. H. CORNFIELD.

Chromatographic separation and determination of fruit acids. A. J. Goudie and W. Rieman, III (*Analyt. chim. Acta*, 1962, **26**, 419—423).—The pH of the juice is adjusted to 4·0 and an aliquot is placed on a Dowex 1-X8 column (acetate form). The column is eluted with a solution containing 0·4M-Na acetate and 2M-acetic acid. Successive fractions contain the sugars, malic acid, tartaric acid and citric acid. The acid content of each fraction is determined by heating with $K_2Cr_2O_2$–H_2SO_4 mixture and measurement of the extinction at 591 mμ. (12 references.) A. J. BENNETT.

Distribution ratios of xanthophylls and carotenes in citrus fruits. U. Gerhardt (*Brauwissenschaft*, 1962, **15**, 172—175).—Pigments of various citrus fruits are extracted and fractionated. In all cases there was an excess of hypophase pigment (xanthophylls with two or more hydroxyl groups). Distribution ratios between phases is suggested as a valuable method of investigating these materials and the effects they are likely to have on the colour of the product. J. B. WOOF.

Hydrophilic colloids in fruit pie fillings. C. E. Kunz and W. B. Robinson (*Food Technol.*, 1962, **16**, No. 7, 100—102).—Starches give a η characterised by resistance to flow (**I**); gums cause a relatively greater resistance to shearing forces (**II**). **I** was measured by the Adams consistometer; **II** by the Brookfield viscometer. In peach and cherry fillings the substitution of hydrophilic gums for starch increased the clarity of the thickened juice in canned and frozen products, but had no effect on colour stability. Effects on deterioration, syneresis, taste, breakdown of fruit are also discussed. E. M. J.

After-cooking discoloration of potatoes. Potassium content of juice in relation to blackening tendency of tissue. E. G. Heisler, J. Siciliano and R. H. Treadway (*Food Technol.*, 1962, **16**, No. 6, 120—124).—A spectrophotometric method was adapted for determining the K constant of potato juice. Samples (44) from 1959 and 1960 crop potatoes from five states east of the Mississippi River were examined for after cooking discoloration and analysed for K. Increasing discoloration was a function of decreasing K content. E. M. J

Potato quality. XIII. Preventing after-cooking discoloration in oil-blanched French fries. O. Smith and C. O. Davis (*Amer. Potato J.*, 1962, **39**, 45—46).—Treatment of potato slices with 2% $Na_2H_2P_2O_7$ in the blancher prevented after-cooking darkening in par-fried French fries and improved the texture and colour uniformity of the finished product. A. H. CORNFIELD.

Comparison of two colorimetric methods for determining reduced ascorbic acid in frozen peas. M. L. Stowell, G. L. Tinklin and D. L. Harrison (*J. Fd Sci.*, 1962, **27**, 347—349).—The Na 2,6-dichloroindophenol (**I**) and the diazotised 4-methoxy-2-nitroaniline methods were compared and very highly significant differences in ascorbic values were obtained, those of method **I** always being the higher. E. M. J.

Consumer preference on a rating basis for almond selections with allowance for environmental and subject-induced correlations. G. A. Baker, M. A. Amerine and D. E. Kester (*Food Technol.*, 1962, **16**, No. 7, 121—123).—Five almond selections judged acceptable by the plant breeders in charge of the breeding programme, were rated by students on a seven-point hedonic scale. If no account is taken of significant correlations between ratings there appear to be no differences between the sexes in almond preference. If correlations are allowed for, very significant differences between almond selections are found with respect to average preference and sex of tasters. There are selection differences between the correlation coeff. that depend on the sex of the tasters. E. M. J.

Non-alcoholic beverages

Determination of activity of polyphenol-oxidase. I. Application of colorimetric methods. II. Polarographic method. W. Heimann and S. Andler (*Z. LebensmittUntersuch.*, 1962, **117**, 121—129, 203—209).—I. On critical examination these methods are judged to be unreliable. (18 references.)

II. The titrimetric, chronometric and manometric methods fail to give satisfactory results. The polarographic method described gives reasonably accurate results for solutions of the purified oxidase, but not for the oxidase as determined directly in natural fruit or vegetable juices. The apparatus comprises a calomel and a dropping Hg electrode. The base solution consists of a phosphate-oxalate-citrate buffer solution at pH 5 which has been saturated with air and which contains small amounts of catechol, quinol, ascorbic acid and gelatin. With this solution the enzymic catalytic reaction proceeds at a constant rate. The determination is based on the observation that the reduction of O_2 proceeds in two steps: (*a*) to H_2O_2 ($E_{\frac{1}{2}} = -0{\cdot}12$ V) and (*b*) to H_2O ($E_{\frac{1}{2}} = -1{\cdot}0$ V); with the use of a saturated calomel electrode, the measurements are best made at −0·5 and −1·35 V. (15 references.) P. S. ARUP.

Detection of the coloration of fruit juice drinks and lemonades by sugar colour. E. Benk (*Riechstoffe u. Aromen*, 1962, **12**, 205—206).—Additions of caramel (**I**) to citrus fruit juices or their conc. syrups or drinks, are detected and determined by colour reactions given by the 5-hydroxymethylfurfural (**II**) in **I**. For qual. purposes, the resorcin–fuming HCl reaction is applied, and for quant. purposes the *p*-toluidine/barbituric acid reaction; colour with this reagent is determined by the extinction coeff. with filter S 55 E. H. L. WHITEHEAD.

Stabilisation of fruit juices by sorbic acid. J. Verrier (*Chim. et Industr.*, 1962, **87**, 631—636).—A simple and economic process for the preservation of grape juice comprises centrifugation and filtration of the juice, addition of 400 mg. of sorbic acid and 200 mg. of ascorbic acid per l. of juice, and, in case of accidental aeration, of 50 mg./l. of SO_2, and conservation at room temp. out of contact with air. Laboratory results have been confirmed by large-scale tests, involving 200 l. during 6 months. M. SULZBACHER.

Polarographic determination of lead in carbonated beverages. P. Sanz Pedrero and E. Fernández de Valderrama (*An. Bromatologia*, 1962, **14**, 9—24).—Available methods for the determination of traces of Pb in foodstuffs are reviewed and a rapid polarographic method is described. Results obtained on 30 samples of soda water on sale in Madrid showed Pb contents ranging from 0·04 to 5·3 p.p.m. Only 5 of 27 siphons analysed had Pb contents lower than the generally recommended limit of 0·3 p.p.m., and the overall mean was 1·25 p.p.m. All siphons with metal tops contained >0·7 p.p.m., those with plastic tops <0·7 p.p.m. Three stoppered bottles had Pb contents of 0·04, 0·07 and 0·65 p.p.m. respectively. Estimated daily consumption in Madrid is approx. 1 l. per head per day, hence this survey indicates a considerable public health hazard and urgent governmental action is urged. (63 references.) E. C. APLING.

Antibacterial additives; identification and determination of the sodium salt of 3-propionyl-6-butylpyran-4-one-2-carboxylic acid in non-alcoholic beverages. L. Polzella (*Boll. Lab. chim. provinc.*, 1961, **12**, 472—478).—A reaction for the detection of Na 6-butyl-3-propionyl-4-oxopyran-2-carboxylate (a preservative) in non-alcoholic beverages consists in extracting the compound from the acidified test solution with $CHCl_3$, adding water, neutralising, treating with alcohol and a drop of $FeCl_3$ solution, and shaking, whereupon an intense yellow colour is produced in the org. layer. Quant., the absorption of the $CHCl_3$ extract is measured at 310 mμ and the concn. of the compound determined with reference to a standard calibration curve. Na benzoate or *p*-hydroxybenzoic esters do not interfere. 0·5 mg. may be detected. L. A. O'NEILL.

Tea, coffee, cocoa

Consumption of coffee and coffee substitutes in Swiss mountain districts. R. Develey (*Mitt. Lebensm. Hyg., Bern*, 1962, **53**, 51—90).—A survey is presented of the consumption in nine valley districts. The high coffee consumption observed in some localities probably arises from the need to supplement the niacin content of the local diets. The physiological effects of coffee and the economic aspects of its consumption are examined. No harmful effects are observed in connexion with high coffee consumption. (49 references.) P. S. ARUP.

Variability of the niacin content in coffee. A. Carvalho (*Nature, Lond.*, 1962, **194**, 1096).—Coffee can be an important source of niacin and so the effect of various growth conditions on the level is described. Shaded environment reduces the concentration considerably and sun-dried beans contain less than those dried in parchment. Selection of strain is also an important factor. J. B. WOOF.

Roasting of some grades of coffee and their blends. C. P. Natarajan, R. Balakrishnan Nair, C. S. Viraktamath, A. Balachandran, D. S. Bhatia and V. Subrahmanyan (*J. sci. industr. Res.*, 1962, **21D**, 116—118).—Various grades of Arabica and Robusta were examined for finishing temp., colour, roasting loss, water extract and organoleptic quality. Since these properties vary widely, recommended finishing temp. are given for each grade. E. C. DOLTON.

Rheology of chocolates. I. The flow equation of N. Casson and its application to rheometry of melted chocolates. W. Heimann and A. Fincke (*Z. LebensmittUntersuch.*, 1962, **117**, 93—103).—The results of experiments in a rotary viscometer at varying r.p.m. show that the linear relationship expressed in the equation between the

sq. roots of the shearing strain and stress holds good with sufficient accuracy for molten chocolate at 37·8°, but not for molten milk chocolate or cacao mass. (18 references.) (See col. 32 above.)
P. S. ARUP.

Coffee extract. General Foods Corp. (Inventors: G. Franck and H. Guggenheim) (B.P. 873,792, 14.12.59).—A conc. coffee extract is produced by forming coffee meal (obtained by expressing or extracting oil from coffee beans) into pellets, distributing the pellets throughout ground roasted coffee, then extracting sol. coffee solids from the mixture with water. F. R. BASFORD.

Milk, Dairy Products, Eggs

Physico-chemical properties of milk. XI. Interfacial tension of milk in *para*-cymene. Balwant Rai Puri and Sat Parkash (*Indian J. Dairy Sci.*, 1962, **15**, 28—38).—Determination of the interfacial tension of over 500 samples of milk in *p*-cymene showed that average values for buffalo and cow milk are similar but that for goat milk is slightly higher. Average values for milk of a particular species did not differ significantly over the seasons of the year. Interfacial tension was markedly influenced by temp., the values increasing with rise from 5 to 25°. Storage at 20—25° had no effect for up to 8 h. (goat milk) or 10 h. (cow and buffalo milk) but thereafter a significant decrease occurred. Abstraction of fat had little effect. Heating to 60° for 10 min. had no effect but heating to 80° for 10 min. resulted in 2% decrease. Addition of water up to 5% caused a reduction in interfacial tension; further addition resulted in an increase. This provides the basis for a simple qual. test for distinguishing genuine from diluted milk. (25 references.) M. O'LEARY.

Identification of κ-casein in zone electrophoresis. J. M. Neelin (*Canad. J. Biochem. Physiol.*, 1962, **40**, 693—695).—By comparison of the results obtained with acid casein and κ-casein, the 'K-zone' was identified in one- and two-dimensional starch gel electrophoresis. Gel media contained either Na veronal and urea, Na veronal alone or Na veronal and $CaCl_2$. S. A. BROOKS.

Caesium-137 in dried milk from Southern England (1959–61) E. M. R. Fisher and D. H. Peirson (*U.K. Atomic Energy Authority Rep.*, 1962, A.E.R.E-M 985, 5 pp.).—^{137}Cs was assayed in dried milk samples from the area of the peak at 0·662 MeV produced by the short-lived daughter ^{137m}Ba and the natural K from the ^{40}K peak at 1·46 MeV. Results are expressed as pico-curies of ^{137}Cs per g. of natural K and this shows that the ^{137}Cs of dried milk has decreased generally with that in rainfall from 1959 to 1961 and agree in general with an independent assessment during the same period.
J. W. TAYLOR.

Antimicrobial agent of aged surface-ripened cheese. II. Sources and properties of active principle(s). N. Grecz, G. M. Dack and L. R. Hedrick (*J. Fd Sci.*, 1962, **27**, 335—342).—*Brevibacterium linens* in the brown bacterial surface smear of the cheese was the principal source of the antimicrobial agent(s) (**I**). Yeast (*Candida* type) and other micro-organisms contributed minor antimicrobial activity. The **I** of aged Liederkranz cheese was (i) dialysable through cellulose casing, (ii) adsorbed on Norit A at pH 3, (iii) stable in acid and alkaline solutions ranging from pH 2·0 to 12·0 at 2—4°, (iv) destroyed within 20 h. in alkaline solution at pH 12 at room temp., (v) in aq. solution stable to heating at 121° for 10 min. at acid pH (<5·0) but showed decreased activity at pH 5·0 to 8·5, (vi) in agar medium of pH 6·8–7·0 activity remained after heating at 121° for 45 min., (vii) was sol. in water, methanol, ethanol, butanol, slightly sol. in acetone and insol. in ethyl ether, CCl_4, $CHCl_3$ and Et acetate, (viii) inhibited growth of many Gram-positive and Gram-negative bacteria, yeasts and moulds, and (ix) was related to antibiotic from *B. linens* but distinct from nisin. E. M. J.

Eggs from hens on diets differing in fat content. R. Jordan, G. E. Vail, J. C. Rogler and W. J. Stadelman (*Food Technol.*, 1962, **16**, No. 6, 118—120).—Three groups of White Leghorn hens were fed diets containing the same ratios of proteins, minerals and vitamins to calories; one diet contained no added fat (**I**), one was supplemented with 10% maize oil (**II**), and the third with 10% beef tallow. The I val. of lipids from eggs from the **II** group increased in contrast with that for **I** and **III**. Cakes made from eggs of group **II** were larger in vol. than those of the other groups; the colour of the yolks of these eggs and of the cakes was a much lighter yellow for **II** group. (18 references.) E. M. J.

Influence of diet and husbandry on the nutritional value of the hen's egg. J. B. M. Coppock and N. W. R. Daniels (*J. Sci. Fd Agric.*, 1962, **13**, 459—467).—The importance of environmental (free range, deep litter and battery), dietary and age factors of the hens is described. In relation to the fatty acid composition of the egg, neither the method of husbandry nor a dietary regime without access to grass, produces eggs so significantly different in essential fatty acid content that eggs produced by any modern system can be said to contribute to the increase in human atherosclerosis. It was shown that arachis and maize oil with a high linolenic acid content are atherosclerogenic in rats in presence of hypercholesterolaemic agents, e.g., cholesterol, cholic acid and thiouracil. (15 references.)
E. M. J.

Effect of iron on the *Pseudomonas* spoilage of farm-washed eggs. J. A. Garibaldi and H. G. Bayne (*Poultry Sci.*, 1962, **41**, 850—853).—On one farm increasing the Fe concn. of the wash water from 0·4 p.p.m. to 10 p.p.m. increased *Pseudomonas* spoilage from 0·8% to 2·5% after 48 days' storage at 13°. On another farm substituting water containing Fe (0·2 p.p.m.) for that containing 4·8 p.p.m. decreased spoilage from 6·2% to 0·8%. A. H. CORNFIELD.

Edible Oils and Fats

Evolutive [ageing] changes in olive oil under the influence of external factors. J. Spiteri (*Dtsch. LebensmittRdsch.*, 1962, **58**, 155—157).—The measurement of $E_{1\,cm.}$ at 270 mμ is proposed as a rapid criterion for the study of changes in stored olive oil, which are accompanied by changes in absorption due to conjugated diene acids (λ max. 232 mμ) and conjugated trienes and unsaturated ketones (weak absorption at 232 mμ; λ max 270 mμ). Values of E 270 mμ vary from 0·08 to 0·47 for virgin oils, from 0·4 to 1·5 for refined oils, and from 1·58 to 4·4 for refined oils prepared from press residues. In normal ageing, E 270 mμ and % free fatty acids increase together, and consideration of a corrected E value (= E 270 mμ − F.F.A. (as oleic acid, %) × 0·023) is proposed for a sharper distinction between aged virgin oils and refined oils which have been freed from fatty acids. E. C. APLING.

Comparison of several analytical techniques for prediction of relative stabilities of fats and oils to oxidation. W. D. Pohle, R. L. Gregory and J. R. Taylor (*J. Amer. Oil Chem. Soc.*, 1962, **39**, 226—229).—Three methods for predicting the stabilities of oils and fats are compared with crystal modified lard, lard and hydrogenated vegetable oil as test samples, with and without the additions of antioxidants. The methods employed are the Eckey Oxygen Absorption, a modified A.S.T.M. Oxygen Bomb and the Active Oxygen methods. The results indicate that the Eckey Oxygen absorption and the Oxygen Bomb methods are more precise than the other method and give results which are more in keeping with those encountered in practice. Results await confirmation of these relationships by actual shelf and organoleptic tests.
G. R. WHALLEY.

Gas–liquid chromatographic fractionation of natural triglyceride mixtures by carbon number. A. Kuksis and M. J. McCarthy (*Canad. J. Biochem. Physiol.*, 1962, **40**, 679—686).—Synthetic and naturally occurring triglyceride mixtures were successfully separated according to their C no. by gas–liquid chromatography in stainless steel columns packed with 60—80 mesh Chromosorb W coated with 2·25% SE-30. Complete and apparently quant. separations of the simple triglycerides from trioctanoin to tristearin were achieved within 40 min. in the temp. range 200—320°. High mol. wt. triglycerides were sometimes lost but no fragmentation occurred.
S. A. BROOKS.

Bis(hydroxynaphthylmethyl)-alkylphenols and their use as antioxidants for fats. U.S. Rubber Co. (B.P. 874,869, 30.12.59. U.S., 5.2.59).—Compounds claimed comprise 2,6-di-(2′-hydroxynaphth-1′-ylmethyl)-4-alkylphenols (the alkyl group containing ⩾10C). They are useful as antioxidants for fats, e.g., butter, vegetable oils, and animal and fish oils. A typical product is 2,6-*di*-(2′-*hydroxynaphth*-1′*ylmethyl*)-4-*t*-*butylphenol*, m.p. 226—228°, obtained in 52% yield by boiling a mixture of 4,2,6-$C_6H_3Bu^t(CH_2{\cdot}OH)_2$, 2-naphthol, benzene and *p*-$C_6H_4Me{\cdot}SO_3H$ for 3 h. F. R. BASFORD.

Hydroxyflavones and their use as antioxidants. National Research Development Corp. (Inventors: T. H. Simpson and N. Uri) (B.P. 875,164, 3.8.56).—Compounds useful as antioxidants (in fatty oils, fats, lipids and inedible matter) comprise 3,7,2′,5′-tetrahydroxyflavones and corresponding 7-alkyl ethers, optionally substituted in the 5-, 6-, 8-, 3′-, 4′- and/or 6′-position by alkyl (and in the case of the 7-alkyl ethers optionally further etherified with an alkyl or aralkyl group in the 2′- and/or 5′-OH), and also 3,7,8,2′,5′-pentahydroxyflavones and their 7- and/or 8- and/or 2′- and/or 5′-alkyl ethers, optionally substituted in the 5-, 6-, 3′-, 4′-, and/or 6′-position by alkyl. They are obtained by oxidation of a suitably substituted chalkone (followed by removal of alkyl groups from alkoxy substituents where desired). A typical product is 3,2′,5′-*trihydroxy*-7-*methoxyflavone* (prep. described), m.p. 228—230°.
F. R. BASFORD.

Meat and Poultry

[A] Chemical and physical changes in meat on heating. II. Influence of heat on state of combination of magnesium, calcium and phosphate in beef muscle. [B] Biochemistry of meat maturing. IV. *Post-mortem* changes in combination of magnesium, calcium, zinc and iron in beef muscle. R. Hamm (*Z. LebensmittUntersuch.*, 1962, **117**, 113—121, 132—138).—[A] In continuation of previous work (cf. *ibid.*, 20), samples of the meat are held at different temp. in the range 20—120° during 30 min. Active liberation of water-sol. Ca and Mg begins and increases rapidly in the range 40—70°; small increases occur at temp. 70—120°. The Ca and Mg in raw and heated meat are more firmly bound at pH 7 than at pH 5·5; their loss entails a decrease in the acidic content of the meat. Most of the liberated Ca and Mg is derived from the structural proteins. The liberation of P as inorg. P begins at temp. >90°, and increases with the temp. Most of the inorg. P is derived from water-sol. constituents of the meat, but at 100—120° some protein-P is liberated. (16 references.)

[B] Immediately after slaughtering the tissue contains little or no free Ca or Zn, but ~60% of the total Mg is in the dissociated form. During the first two days the free Ca, Zn and Mg increase by ~10—20%, after which the free Ca (only) increases slowly. During the first two days the liberated Ca and Zn are derived solely from water-sol. org. constituents, whilst appreciable amounts of Mg are liberated from the structural proteins; during the following five days Ca and Mg (but no Zn) are liberated in moderate amounts from the structural proteins. The muscle-Fe remains bound during the whole period. Probable mechanisms of the *post-mortem* changes are considered. (27 references.) P. S. ARUP.

Comparative studies of meat. VIII. The percentage of fat in the fatty and muscular tissues of steers and the iodine number of the extracted fat, as affected by breed and level of nutrition. E. H. Callow (*J. agric. Sci.*, 1962, **58**, 295—307).—The % fat in the tissues of various joints decreases with breed in the order Shorthorn, Hereford and Friesian, although no such difference in I val. is found in the subcutaneous or intermuscular tissue. A good correlation exists between I val. and % fat in the joint. Certain joints have tissues which give abnormal figures for I val., deep-seated tissues having low values. The % fat is highest in the thorax and pelvis, and lowest in the fore and hindshins, I val. being the opposite. A similar gradient is shown by the subcutaneous and intermuscular tissue of the joints. The rate of fattening is greatest with animals fed on concentrates. M. LONG.

Effects of sonic vibration on tenderness of beef. N. B. Webb, L. J. Bratzler and W. T. Magee (*Food Technol.*, 1962, **16**, No. 6, 124—128).—At the energy level and frequency used (a low-power 10-kc transducer unit) sonic treatments of 20 and 60 sec. did not increase the tenderness for either fresh or frozen steaks (*longissimus dorsi* muscle of utility cow carcasses). When sonic treatment was applied for 10 min. there was slight but significant increase in tenderness and a significant decrease in the drip loss of frozen steaks. Enzyme treatment of frozen steaks caused a significant increase in tenderness and a decrease in drip and total losses. 80° brine (NaCl) couplant significantly increased tenderness, juiciness and flavour scores of fresh and frozen steaks. Cryovac film prevented any significant sonic tenderisation. (16 references.) E. M. J.

Effects of physical conditions on the drying of minced mutton. A. R. Prater and G. G. Coote (*Commonw. sci. industr. Res. Org. Aust., Div. Fd Pres.*, 1962, tech. Paper No. 28, 30 pp.).—Drying of precooked minced mutton by the through-draught process occurred in three phases: constant rate, a transition stage and a period of falling rate. An estimate of the drying time under selected conditions was obtained by use of the appropriate regression equation. Variation of fat content in the fresh meat affected the palatability and texture of the dried mince and had a major influence on the drying rates especially in the final stage. Dried mince of a satisfactory quality and shelf life could be produced when a dry bulb temp. of 160°F was maintained throughout the drying period. E. M. J.

Spectrophotometric estimation of metmyoglobin in frozen meat extracts. J. P. Lane and L. J. Bratzler (*J. Fd Sci.*, 1962, **27**, 343—346).—Frozen meat-water extracts stored under light exhibit a pattern of metmyoglobin formation similar to that in frozen steaks. Fluorescent lights significantly increased the formation of metmyoglobin in the solutions. The increase of metmyoglobin was inhibited by dialysing the extract. Addition of Cu^{2+}, Mn^{2+}, or Ca^{2+} as chloride salts to the dialysed solution did not increase rate of formation of metmyoglobin appreciably, but those of Mg^{2+} and Fe^{2+} and Fe^{3+} contributed to an increase. When the dialysate was concentrated and added to the dialysed residue there was a significant increase in the rate of metmyoglobin formation. (10 references.) E. M. J.

Interpretation of the spectra of meat pigments. I. Cooked meats. II. Cured meats. The mechanism of colour fading. B. G. Tarladgis (*J. Sci. Fd Agric.*, 1962, **13**, 481—484, 485—491).—I. According to quantum mechanical theories the absorption or reflectance spectra of the metal co-ordination complexes are closely related with the electronic configuration of both the co-ordinated metal and the ligands which occupy the fifth and sixth co-ordination positions of the metal ion. The compound responsible for the colour of cooked meats was identified as a high-spin ferric–porphyrin co-ordination complex. The fifth and sixth co-ordination positions of the ferric ion of this compound are occupied by a carboxylate ion of the denatured globin mol. and by water, respectively. This compound is metmyochromogen. (24 references.)

II. The spectra of the pigments of cooked cured ham and of heat-denatured nitric oxide haemoglobin show that the pigments are identical. Both compounds are low-spin ferrous–porphyrin co-ordination complexes, with both the co-ordination positions of the Fe ion occupied by nitric oxide. This compound is nitric oxide myochrome. The spectra of fresh cured ham and those of nitric oxide haemoglobin derived from fresh blood are very similar. They are low-spin ferrous–porphyrin co-ordination complexes. One co-ordination position of the Fe is occupied by globin, the other by nitric oxide. This pigment is nitric oxide haemo(myo)-globin. The mechanism of colour fading is discussed. The prep. of colour-stable cured meats is considered from the theoretical standpoint. (26 references.) E. M. J.

Modified 2-thiobarbituric acid (TBA) method for the determination of malonaldehyde in cured meats. M. W. Zipser and B. M. Watts (*Food Technol.*, 1962, **16**, No. 7, 102—104).—Small amounts of NO_2^- are capable of reducing TBA numbers of rancid meat significantly, and the reduction increases linearly with NO_2^- concentration. During distillation in the TBA procedure nitrosation of the malonaldehyde takes place. The NO_2^- interference is controlled by diazonium salt formation with sulphanilamide to bind the NO_2^- before beginning the TBA test. E. M. J.

Removal of interfering pigments in determining malonaldehyde by the 2-thiobarbituric acid [TBA] reaction. T. C. Yu and R. O. Sinnhuber (*Food Technol.*, 1962, **16**, No. 6, 115—117).—The procedure described is an additional step in the TBA reaction of Yu and Sinnhuber (1957) that permits separation of the 532—535-mμ pigment from the yellow interfering colour by two methods. A corrected malonaldehyde value may then be obtained. A formula may also be derived that allows calculation of the actual malonaldehyde on future or additional samples without chromatographic separation or purification of the pink 535-mμ colour. This extends the TBA–malonaldehyde reaction to such foods as oysters, ham, baked goods and other materials that give interfering pigments. (14 references.) E. M. J.

Freezer burn as a limiting factor in the storage of animal tissue. III. Experiments with liver frozen with and without evaporative weight loss. G. Kaess and J. F. Weidemann (*Food Technol.*, 1962, **16**, No. 7, 125—130).—The loss in wt. necessary to produce a definite intensity of freezer burn (fb), when a sample of frozen liver is stored at −10° at 78, 88 and 97% R.H., increases with rate of freezing when freezing times are varied from 4 to 410 min. When evaporative wt. loss takes place during freezing the effect is greater. The effect of freezing rate is less with high than low fat content. The conditions under which the appearance of fb is delayed until after the appearance of dark discoloration are young age of the animal, low moisture content of the liver and low rate of freezing (either without or preferably with evaporative loss) of livers of low fat content. E. M. J.

Use of diethyl pyrocarbonate as preservative for poultry meat. W. Schmidt-Lorenz (*Z. LebensmittUntersuch.*, 1962, **117**, 231—241).—By steeping shortly after slaughtering in a 0·05% solution of the ester, the storage life of the meat is prolonged by 60—65%. The odour of the ester disappears from the meat during the first two days in storage. Very considerable reductions of the surface bacterial flora are observed after the steeping. In spite of these good results, the use of the ester is not recommended at present. (11 references.) P. S. ARUP.

Bacon products. W. J. Stange Co. (B.P. 876,859, 8.1.58. U.S., 8.1.57).—A stable bacon product with lean and fat parts if produced by comminuting and rendering the bacon (by heating in a kettle with steam at 30—60 p.s.i.), and uniformly dispersing an antioxidant (propyl gallate) in the rendered bacon when the fat portion is in the liquid state (e.g., at 130°F). The product is useful for flavouring purposes. F. R. BASFORD.

Fish

Proteins in fish muscle. XVII. Fractionation of aqueous extracts with zinc acetate. J. R. Dingle, J. A. Hines and J. M. Neelin (*J. Fish. Res. Bd Can.*, 1962, **19**, 591—604).—When aq. extracts of cod and haddock muscle were treated with 0·1M-Zn acetate and KH_2PO_4 buffer the sol. cod proteins remaining were approx. 18%. This fraction consisted mainly of two components present in the original extract. Similar results were obtained from haddock muscle. The two main components were not separated on DEAE cellulose column. The cod fraction contained an unusually large amount of phenylalanine. (11 references.) E. M. J.

Lipase and phospholipase activity in fish skeletal muscle and its relationship to protein denaturation. J. Olley, R. Pirie and H. Watson (*J. Sci. Fd Agric.*, 1962, **13**, 501—516).—The production of free fatty acids (FFA) and/or phospholipid breakdown (**I**) in the denaturation of fish muscle proteins (**II**) during cold storage are discussed. While denaturation of **II** and **I** or FFA production run parallel in the cod, the deduction that the two phenomena are related cannot be extended to other species, viz., lemon sole, halibut and dogfish. There is no evidence for a simple causal relationship between **I** or FFA production and denaturation of **II**. Lipolytic activity during cold storage at −7 and −14° was studied in 11 other species. Phospholipase activity was negligible in the three Elasmobranchs dogfish, nursehound and skate. In all the other species phospholipase was as important as lipase in producing FFA and in the Gadoids and related species FFA were produced mainly by hydrolysis of phospholipids. Freezing and thawing of whole fish cause FFA production. (45 references.) E. M. J.

Reaction of cod actomyosin with linoleic and linolenic acids. F. J. King, M. L. Anderson and M. A. Steinberg (*J. Fd Sci.*, 1962, **27**, 363—366).—The solubility of cod actomyosin is reduced by small concn. of linoleic and linolenic acids. The rate of insolubilisation of this protein depends on the structure of the fatty acid and its concn. and the time of storage. Findings supported the results of Dyer and Fraser (1959) in a study on changes in frozen cod muscle stored at −12° when they found that the actomyosin had become totally inextractable after 30 weeks of storage. Linoleic acid 0·1 mg. per mg. of total sol. protein N, causes insolubilisation of ~½ of extracted fresh cod actomyosin after 4 days at 4°. (21 references.) E. M. J.

Degradation of adenine- and hypoxanthine-nucleotide in the muscle of chill-stored trawled cod (*Gadus collarias*). N. R. Jones and J. Murray (*J. Sci. Fd Agric.*, 1962, **13**, 475—480).—Adenosine triphosphate (ATP) concn. in the muscle of freshly-killed trawled cod was very low compared with that of fish which had been rested and well fed experimentally. Levels of inosine monophosphate (IMP) which derives from deamination and dephosphorylation of ATP were proportionately higher. Additional enzymic cleavage of some of the remaining adenine nucleotide within a day or so of death accounts for the rise in IMP concn. IMP is dephosphorylated to inosine which is cleaved to hypoxanthine and ribose or ribose 1-phosphate. The patterns of degradation of IMP on the two groups of fish were very similar. Stoicheiometry in the reaction sequence was good. IMP is of importance to the technology of flesh foods in connexion with 'meaty' flavour. A course of degradation of nucleotide in chill-stored cod muscle is outlined. (41 references.) E. M. J.

Acid-soluble phosphorus compounds and free sugars in fish muscle and their origin. H. L. A. Tarr and M. Leroux (*Canad. J. Biochem. Physiol.*, 1962, **40**, 571—589).—The distribution of sugar phosphates and nucleotides in muscles of freshly killed fish and in muscles after several days at 0° was studied by an ion-exchange system. Extreme quant. variations were found. Various metabolic pathways for these compounds are suggested by the results. (61 references.) S. A. Brooks.

Conversion of free fatty acids of cod oil to methyl esters *in situ*. R. G. Ackman, L. R. Gallay, P. M. Jangaard and M. L. Hughes (*J. Fish. Res. Bd Can.*, 1962, **19**, 605—614).—The conversion of free fatty acids (FFA) to esters of the lower alcohols and the competing reactions involved are discussed. The problem of removing the water formed during the esterification was solved by application of 2,2-dimethoxypropane (**I**) to the *in situ* conversion of the FFA of cod oil. **I** in presence of a suitable acid catalyst (methanolic HCl) serves as a water scavenger and a source of methanol for the esterification. The fatty acid content is reduced to ~1%. The purification and stabilisation of the product are described. E. M. J.

Effect of tetracycline antibiotics on objective and subjective fish quality tests. J. W. Boyd and B. A. Southcott (*J. Fish. Res. Bd Can.*, 1962, **19**, 615—618).—Chlortetracycline and oxytetracycline significantly suppress the reduction of trimethylamine oxide to trimethylamine in lingcod muscle during storate at 0 and 4° but under conditions of the tests the bacterial population was not correspondingly reduced. A reasonable degree of agreement was obtained between viable bacterial count, trimethylamine content and organoleptic score from tests on control samples. E. M. J.

Fish liver paste. A Guttmann (*Trade News*, 1961, **13**, No. 8, 6—7, *Stud. Fish. Res. Bd Can.*, 1961 [1962], No. 677).—As a vitamin food supplement fish livers compare favourably with mammalian liver and, for some inorg. and org. compounds, with, e.g., pork liver. The enormous quantities of residue from the fish oil industry could be used to make liver paste with high nutritive value as a sandwich spread. Details are given of the prep. of residue from 100 kg. of minced fresh cod livers. The residue was then neutralised, wheat flour and vegetable oil were mixed with it and salt and spices added. The resulting paste was generally accepted and considered very palatable. E. M. J.

Moisture in fish blocks processed from very fresh fish. W. J. Dyer and D. I. Fraser (*Canad. Fisherman*, 1961, Aug., *Stud. Fish. Res. Bd Can.*, 1961 [1962], No. 685).—The difficulty associated with 'wet' fish blocks used for the prep. of fish sticks is caused by the drip which accumulates in the trays and is frozen into the blocks. The moisture arises from two sources, (*a*) that left on the fillets after washing or dipping into antibiotic solutions etc., (*b*) that leaking from inside the fish. The physical and chemical changes taking place after death in rigor mortis are discussed. When the pH or acidity of the muscle is changed by lactic acid formation, the moisture-binding capacity of the protein is reduced and drip formation may result. The chemical processes were observed at 32, 50 and 75°F in rested freshly killed cod. The changes were half completed in 12, 6 and 1 h., respectively. E. M. J.

Influence of selected preparation procedures on flavour and aroma of fish. R. E. Baldwin, D. H. Strong and J. H. Torrie (*Food Technol.*, 1962, **16**, No. 7, 115—118).—Flavour and aroma scores as tested by taste panel on three species of fish were not sufficiently altered by the selected techniques of skinning, boning or frying to suggest practical ways of modifying or eliminating any undesirable flavours already present in the fish. (16 references.) E. M. J.

Giant scallops in Newfoundland coastal waters. H. J. Squires (*Bull. Fish. Res. Bd Can.*, 1962, No. 135, 29 pp.).—Exploratory fishing for *Placopecten magellanicus* Gmelin during the summers of 1957 and 1958 is reported. Data on meat counts, maturity and temp. effects are given. (12 references.) E. G. Brickell.

Radiation-pasteurised shrimp and crabmeat. D. J. Scholz, R. O. Sinnhuber, D. M. East and A. W. Anderson (*Food Technol.*, 1962, **16**, No. 7, 118—120).—Quality determinations showed that storage life of crabmeat irradiated at 0·25 Mrad was extended 3—4 weeks compared with 1 week for unirradiated samples held at the same temp. A dose of 0·50 Mrad extended shelf life for 5 weeks. Shrimp irradiated at 0·50 and 0·75 Mrad remained in good condition for the 18-week storage period. No viable micro-organisms were found, decomposition products were slight as measured by chemical tests. Extension of shelf life was threefold. Irradiation flavour of both declined during storage. (11 references.) E. M. J.

Spices, Flavours, etc.

Systematics of differential tests in organoleptic analysis. Luis Hidalgo and Manuel R. Candela (*Bol. Inst. Invest. agron., Madr.*, 1961, **21**, 385—404).—The statistical basis of three systems of organoleptic testing, paired, triangular and scalar (hedonic), are fully described, and tables of significance for use in the selection of tasters and for establishing differences between samples are reproduced for the paired and triangular systems. (26 references.) E. C. Apling.

Methods for producing aroma concentrates. A. M. Burger (*Riechstoffe u. Aromen*, 1962, **12**, 207—209).—Discussions are given of processes for making aroma concentrates from fruit products by extraction with org. solvents followed by removal of the solvent, also of the problems involved in removing the solvent without detriment to the aroma compounds. The solvent(s) should have similar polarity to the aroma compounds for good extraction, and be miscible with water at the operating temp. (these matters are fully discussed). Most (80%) of the solvent can be removed by distillation under normal pressure, thereafter the (stage-wise) distillation must be conducted under vacuum with a climbing film evaporator without true (bubble-forming) boiling in the film: a rotary laboratory apparatus for this purpose is described. Pure extracts of the total aroma compounds can also be obtained by steam distillation of the fruit product under vacuum. H. L. Whitehead.

Survey of the ethereal citrus oils. H. E. Swisher (*Riechstoffe u. Aromen.*, 1962, **12**, 194—196).—Descriptions and discussions are given of the compositions, *qua* individual compounds, of the ethereal oils of various citrus fruits (oranges, grapefruit, etc.), and of the methods employed for effecting the analysis of the oils: also of the various aldehydes, etc. that give the characteristic aroma, and the various carbonyl compounds (aldehydes and esters) and alcohols that give the characteristic flavours. H. L. WHITEHEAD.

Comparative examination of [methods for] determination of ethereal oils in condiments. H. Hadorn (*Mitt. Lebensm. Hyg., Bern*, 1962, **53**, 43—50).—The standard deviations shown by collaborative analyses of black pepper and cloves show appreciable differences as between four different laboratories. On the whole, the deviations shown by results obtained by the diffusion method of Hadorn *et al.* (cf. *Anal. Abstr.*, 1954, **1**, 3120) are greater than those shown by the results of the Flück distillation (AOAC) method. Comparatively low standard deviations for the Hadorn method are, however, reported by a laboratory in which this method has been in use during 6 years, viz. ±0·08 for black pepper and ±0·45 for cloves (13 samples of each). P. S. ARUP.

Standardisation of vegetable drugs. IV. Comparison of methods for the determination of essential oil. P. Duquénois (*Ann. pharm. franç.*, 1962, **20**, 244—256).—The various methods used to determine essential oils in vegetable matter are reviewed. Gravimetric and titrimetric methods are rejected as being less relevant to industrial methods of extraction, and three methods of steam distillation followed by measurement of the volume of non-aqueous distillate are compared. Three methods are used for the comparison of time taken for complete distillation, the advantages of fine powdering of the materials, testing the advantages of addition of glycerol to raise the boiling temp., the design of the volume-measuring part of the apparatus, and the nature of the non-aqueous solvent to be added when the oil is more dense than water or is viscous. The B.P. method is considered as good as the others and is recommended. E. J. H. BIRCH.

Colouring matters

Artificial food colours. VIII. Characteristics, properties, spectrophotometry and chromatography of some prohibited water insoluble [colours]. L. Villanúa, A. Carballido, R. García Olmedo and M. T. Valdehita (*An. Bromatologia*, 1962, **14**, 51—74).—Tabulated data are given for 11 water-insol., ethanol-sol. dyes (red, oranges and yellows), included in the Ascona prohibited list, viz.: Methyl Red (C.I. 13·020); Sudan III (C.I. 26·100), Sudan IV (C.I. 26·105); Parafuchsine (C.I. 42·500); Sudan I (C.I. 12·055); Sudan II (C.I. 12·140); Orange SS (Orange BN); Yellow AB (C.I. 11·380); Yellow OB (C.I. 11·390); *o*-aminoazotoluene (C.I. 11·160) and *p*-dimethylamino-azobenzene (C.I. 11·020). Green's technique (reduction with Zn) is preferred to Rota's technique ($SnCl_2$) for the decolorisation of reducible dyes. (13 references.) E. C. APLING.

Colour in capsicum species. I. Variation of colour in paprika and its extracts. II. Method of colour determination. J. Sancho, F. Navarro and **II** A. Serna (*An. Bromatologia*, 1962, **14**, 25—34, 35—50).—I. The effects of temp., light, solvent composition, etc., on estimation of carotenoid pigments with min. extraction of water-sol. pigments are studied. Max. effects of light and temp. were shown with ethanol extraction, and most reproducible results were obtained by extraction with iso-propanol for 48 h. at room temp. On storage for 1 year, loss of pigments in whole fruit was reduced by storage in the dark and by refrigeration at 5°. Preliminary results suggest that antioxidants have no effect and that colour losses are increased in the presence of bactericides; possibly the presence of *Bacillus subtilis* contributes to colour retention.

II. A standard method, employing transmission measurements at a single wavelength (460 mμ), but eliminating instrumental differences is proposed. Measured transmissions are interpreted in terms of colour purity from a calibration graph prepared from transmission measurements made with the same instrument on standard solutions of K_2CrO_3 and $CoCl_2,6H_2O$ for which colour purities are tabulated. Results on 122 samples obtained with a spectrophotometer and two colorimeters using blue filters showed very close agreement (deviations generally <0·5) with true colour purity. E. C. APLING.

Polyene carboxylic acids and esters thereof. F. Hoffmann-La Roche & Co. A.-G. (Inventors: W. Guex, O. Isler, R. Ruegg and G. Ryser) (B.P. 875,713, 28.8.58).—Compounds claimed have the formula R·CH:CH·CMe:CH·CH:CH·CMe:CH·CH:CH·[CH:CR′]$_n$·CO_2X [R is 2,6,6-trimethylcyclohexenyl; *n* is 0—3; R′ (reading from left to right) is Me and is present only in fragments in brackets corresponding to the first and third extension of the chain; X is H or alkyl]. A detailed example describes the prep. of 17-(2,6,6-*trimethylcyclohexenyl*)-2,6,11,15-*tetramethylheptadeca*-2,4,6,8,10,12,14,-16-*octaen*-1-*oic acid*, m.p. 189—190° (*Me ester*, m.p. 136—137°). The products are food colours. F. R. BASFORD.

Food Processing, Refrigeration

Drying without heat. Anon. (*Food Engng*, 1962, **34**, No. 7, 84—85).—Non-heated air with 3% R.H. is used to give an excellent end product. As an example, a tomato concentrate (28—30% solids) is dried to a powder with 4% moisture by spraying into a 50 × 220 ft. tower against a current of low-velocity air at <86°F. Volatiles are retained. The droplets take 90 sec. to fall through the tower and are then recirculated. The process can also be applied to cheese (0·7—2·0% final moisture), and for prep. of dried cream and butter and whole milk powder (2·3—3·1% moisture). C. V.

Preservation of quality of thin-skinned potatoes by moderate X-ray treatment. A. Berger and H. Hansen (*Z. LebensmittUntersuch.*, 1962, **117**, 215—225).—In experiments with two varieties it is shown that sprouting can be inhibited during 34 weeks by exposing the fresh potatoes (kept in rolling motion) to a lower dosage than previously considered necessary, viz. 5000 r at 60 kV. In comparison with treatments at 10,000—15,000 r, this treatment results in a better appearance of the skin, a better flavour, and avoids losses of ascorbic acid and abnormalities in metabolism during storage. P. S. ARUP.

Feasibility in the United Kingdom of a radiation process for the inhibition of sprouting in stored potatoes. F. J. Ley, I. D. Clarke and W. G. Burton (*U.K. Atomic Energy Authority Rep.*, 1962, A.E.R.E. R/3977, 22 pp.).—The potato industry in the U.K. is reviewed in terms of acreage, varieties, distribution, quantity stored and consumption. Sprouting suppression can save up to 1·4% of the total crop stored; possible methods include ventilation, refrigeration, chemical methods, etc., for which detailed cost assessments are given. Details are given of the design and economics of a plant for the inhibition of sprouting with a 10^4 rad source for quantities of 2—90 × 10^3 tons/year. The assessment indicates irradiation to be expensive in relation to other procedures even at the highest quantities treated by a factor of 1·5 to 9 times and likely developments do not appear capable of substantially reducing these costs in the U.K.; the technique may be economic in other countries where losses are higher. (12 references.) J. W. TAYLOR.

Frozen fruit pies. K. Kulp and W. G. Bechtel (*Food Technol.*, 1962, **16**, No. 7, 104—106).—The time of freezing of fruit pies, up to 28 h., had no effect on the quality of the crust or filling. For rapid freezing, low temp. and forced air circulation are necessary and frozen pies should be stored at 0°F or below. Evidence of deterioration during the experimental storage periods of 0° and −20°F was the soaking of the bottom crust; after 16 weeks it was slight for apple pies, and moderate for cherry and most severe for boysenberry pies. (10 references.) E. M. J.

Free radicals in lyophilised food materials. K. A. Munday, M. L. Edwards and G. A. Kerkut (*J. Sci. Fd Agric.*, 1962, **13**, 455—458).—In fresh heart muscle at 77°K the free-radical concentration was between 2·0 and 5·2 × 10^{15} per g. of tissue; in food materials dried by the accelerated freeze drying (A.F.D.) process, A.F.D. minced beef 8·25 × 10^{15}, A.F.D. herring 6·47 × 10^{14}; A.F.D. maize 1·78 × 10^{14}; A.F.D. rat cake 1·30 × 10^{14}. No free radicals were found in minced beef or maize before A.F.D. processing or in quick-frozen (−15°) minced beef stored some weeks. A.F.D. tissues have a higher concn. of free radicals than fresh tissue. The free-radical concn. is increased by heating to 100° and is higher in foods with a high lipid content. Distilled water (0·1 and 0·5 ml.) added to A.F.D. samples of minced beef (~0·2 g. dry wt.) caused a decline in free-radical concn. and in all cases it was finally abolished. (11 references.) E. M. J.

Packaging

Effects of illumination on film of regenerated cellulose. W. B. S. Bishop, F. A. Lee, D. G. Saville and J. F. Williams (*Food Technol. Aust.*, 1962, **14**, 202—203, 205, 207, 240).—Printed cellulose films of the M.S.A.T. type used for wrapping fats liberated, in presence of light, various volatile substances identified as methyl ethyl ketone, diacetyl, ethanol, acetates etc., and a sulphurous odour. H_2S produced in 1½ h., in light, from nitrocellulose lacquer coated film (three types), gravure printed, was 1·5, 2·0 and 3·0 p.p.m., respectively. Food taste can be contaminated by contact with printed food wraps, with solvents, plasticisers, inks, coatings. Food so wrapped should be protected from direct sunlight. E. M. J.

Determination of antioxidants in polyethylene by thin-layer chromatography. R. F. v. d. Heide and O. Wouters (*Z. Lebensmitt-Untersuch.*, 1962, **117,** 129—131).—The antioxidant is extracted by steeping the sample in Et_2O (peroxide free) during 24 h. in darkness with occasional shaking. After evaporation of the Et_2O aliquots of a solution of the residue dissolved in Et_2O are chromatographed on a SiO_2-gel thin layer with light petroleum + 10% of EtOAc as solvent. Data for eight commercial antioxidants include R_F values and colour reactions obtained by spraying the chromatograms with 2,6-dichloroquinone-chlorimide (followed by aq. borax) or with a solution of diazotised *p*-nitroaniline. P. S. ARUP.

Mould-growth resisting compositions. Mosinee Paper Mills Co. (B.P. 876,069, 16.5.58. U.S., 23.5.57).—Paper (especially when used for packaging of food) is rendered mould-resistant by coating with a composition containing a product obtained by interaction of biphenyl (I) with a substituted monohydric phenol (II), e.g., a phenylphenol (preferably *o*-phenylphenol), and a rosin amine (1 mol.), e.g., dehydroabietylamine. The composition may also include a water-resistant binder and microcrystalline wax.
F. R. BASFORD.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Nutritive value of canned fish: effect of canning, storage and antioxidants. P. L. Sawant and N. G. Magar (*J. sci. industr. Res.*, 1961, **20D,** 313—316).—The effect of canning and storage in the presence and absence of antioxidants, on the nutrients in black and white pomphrets and prawns was studied. Canning denatures the proteins and results in significant loss in thiamine; riboflavin, niacin and the amino-acid contents of the fish are not adversely affected. On storage, there is a marked loss of lysine, arginine, methionine and tryptophan. Riboflavin and niacin are more stable than thiamine. The extent of the losses increases with use in storage temp. Nordihydroguaiaretic acid and ascorbic acid when present together prevent discoloration of the canned product.
K. M. H.

Effect of dietary changes on fatty acid composition of normal human depot fat. K. J. Kingsbury, T. D. Heyes, D. M. Morgan, C. Aylott, P. A. Burton, R. Emmerson and P. J. A. Robinson (*Biochem. J.*, 1962, **84,** 124—133).—When ethyl arachidonate, a cod-liver oil fraction rich in C_{20} pentaenes and C_{22} hexaenes, maize oil and cod-liver oil, are substituted singly for the equiv. amount of fat in a controlled diet, and are given separately during periods of 14 days to human subjects, the dietary polyunsaturated acids appear rapidly in the plasma lipids but only sparsely in the depot fats. There are marked individual changes in the monoene and saturated fatty acids of the plasma and depot fat, and they appear to be unrelated to the type of fat in the diet. The fatty acid changes of the plasma and depot fat are often quite different. It is concluded that the fatty acid composition of the body is always in a state of change often unrelated to the dietary fat. Changes in the lipids of one body compartment are not reliable indications of changes in another. J. N. ASHLEY.

Conversion of ^{14}C-labelled 'glucose cycloacetoacetate' into L-ascorbic acid in albino rats. M. L. Belkhode and M. C. Nath (*Biochem. J.*, 1962, **84,** 71—74).—After intraperitoneal injection of [1-^{14}C] glucose cycloacetoacetate into chloretone-treated rats, the ascorbic acid excreted in the urine has 64—71% of the ^{14}C in C-6 and 8—10% in C-1. Where a similar experiment is carried out with [6-^{14}C] glucose cycloacetoacetate, the ascorbic acid recovered from the urine has 66—68% of the ^{14}C in C-1 and 8—11% in C-6. The results show that during the conversion the glucose moiety remains intact and simultaneously undergoes inversion. (24 references.)
J. N. ASHLEY.

Flash desolventising defatted soya-bean meals washed with aqueous alcohols to yield a high-protein product. G. C. Mustakas, L. D. Kirk and E. L. Griffin, jun. (*J. Amer. Oil Chem. Soc.*, 1962, **39,** 222—226).—Wetted soya-bean protein meals are washed with aq. alcohols to give improved flavour and increased protein content. A vapour-type flash solvent removal system is employed to remove the excess solvent after extraction. Protein enrichment of the meal from 50 to 75% is achieved by washing with 50 to 75% aq. solution of methanol, ethanol or isopropanol. After solvent removal, the residual alcohol content of the flakes is 0·25 to 1%, methanol being the most easily removed. A two-stage flash system is also employed, although there is some evidence for the retention of the alcohol by the flake by absorption or by H-bonding. The resultant free-running flakes have a protein content of 72—77%, N solubility index of 4—16, and a water absorption value of 328—410%.
G. R. WHALLEY.

Method for determination of cystine plus cysteine in proteins. J. C. Fletcher and A. Robson (*Biochem. J.*, 1962, **84,** 439—444).—The protein is hydrolysed in presence of [^{35}S]cystine and the cystine in the hydrolysate is isolated by chromatography, and its sp. activity is determined. Then cystine S plus cysteine S is equal to the total activity added before hydrolysis divided by the sp. activity of the isolated cystine. The method is based on results obtained with wool keratin, but has given good results with insulin, lysozyme and bovine plasma albumin. It is time-consuming and is unsuitable for routine work. It could be used to standardise, empirically, other methods for determination of cystine, such as the phosphotungstic acid method, which are suitable for routine and serial analysis. (29 references.) J. N. ASHLEY.

Amino-acid composition of groundnut protein isolates. M. N. Satyanarayana, M. V. L. Rao, M. Srinivasan, A. Sreenivasan and V. Subrahmanyan (*Food Sci., Mysore,* 1962, **11,** 133—135).—Determination of the essential amino-acid contents of protein isolates from groundnut kernels and edible quality press cake, dried by various methods at temp. ranging from 55 to 115°, showed that none of the processing methods employed significantly decreased the nutritive value of the products. (17 references.)
M. O'LEARY.

Methionine content of some South Indian foods. K. Dakshinamurti (*J. Fd Sci.*, 1962, **27,** 367—369).—Methionine content of foodstuffs common in South India is tabulated. Two methods, one based on the McCarthy and Sullivan colour reaction and the other on filter paper disk chromatography were used, the former giving slightly higher values. As sources of methionine vegetables are inferior to cereals, pulses are the richest. (12 references.)
E. M. J.

Amino-acids in sewage during treatment. B. Meera Bai, C. V. Viswanathan and S. C. Pillai (*J. sci. industr. Res.*, 1962, **21C,** 72—76).—Samples of sewage were treated in comparative tests by chemical coagulation with alum, sedimentation, septic tank (anaerobic) and activated sludge (aerobic) processes, and the amino-acid contents and distribution in the sludges and effluents from each process were determined. The sludge from the aerobic process contained the most org. matter and N, and the effluent, the least, being free from amino-acids and almost free from NH_3. The distribution of the amino-acids differed in the different processes, the sludge from the chemical treatment being rich in glycine, serine, threonine and arginine; that from sedimentation was similar. The anaerobic sludge was much poorer in all amino-acids and the aerobic rich in all the amino-acids, particularly in the leucines. It is suggested that the aerobic sludge could be used as a feed supplement for chicks and pigs. (16 references.) J. I. M. JONES.

Tocopherol and ubiquinone changes during germination. N. K. Garg and C. R. Krishna Murti (*J. sci. industr. Res.*, 1962, **21C,** 103—105).—Changes in the concn. of total lipids, ubiquinone, plastoquinone and tocopherols during different stages of germination of *ragi* (*Eleusine coracana*) and *thinai* (*Panicum italicum*) were studied. The degree and magnitude of changes suggest that saponifiable lipids are being consumed and that ubiquinone, plastoquinone and tocopherols undergo transformation during germination. (13 references.) S. A. BROOKS.

Protein food products. Unilever Ltd. (Inventors: A. Hurst, B. M. Gibbs and B. D. Hemmings) (B.P. 874,537, 29.6.57).—A cheese-like product is prepared from groundnut protein by adding fat to an aq. suspension or solution of groundnut protein; homogenising the mixture; heating to ⩽80° (before or after adding the fat); then precipitating curd by addition of acid; cutting the resulting curd (at pH 5·8—6·3); and draining, pressing and ripening it. Preferably, lactic acid is used for pptn. of the curd and it may be formed *in situ* (after the heat treatment) by fermentation produced by a starter (e.g., a sucrose fermenting strain of lactic streptococcus) in presence of a suitable carbohydrate (sucrose) and, if desired, rennet. F. R. BASFORD.

Proteinaceous material. Wilson & Co. Inc. (B.P. 875,596, 24.11.58, U.S., 29.11.57).—An aq. solution of material formed by hydrolysis of collagen-containing matter (bones, skin, sinews) is treated (at −17° to +20°) with a water-sol. protein-pptg. agent (monohydric alkanol, low-mol. ketone), to effect pptn. of a non-gelling hydrolysed protein, suitable for use in the food, cosmetic and pharmaceutical industries. If desired, the ppt. may be purified (freed from colour, etc.) by treatment in aq. solution with a bleaching agent, optionally after destroying any peroxidase present (by heating at 85—100° during 30—60 min.). F. R. BASFORD.

Unclassified

Effect of sodium chloride on the growth of certain yeasts of marine origin. S. R. Ross and E. O. Morris (*J. Sci. Fd Agric.*, 1962, **13,**

467—475).—The ability to tolerate a certain concn. of NaCl can be affected by the nature of the N source; e.g., fish extract caused an increase in tolerance compared with $(NH)_2SO_4$ whereas urea caused a decrease. The time required for visible growth to appear increases as the concn. of NaCl in the medium is raised. When raised to 8% the lag phase increases and duration of the exponential phase decreases while rate of growth shows little change. There is correlation between the halo-tolerance of particular species isolated from different marine locations. *Debaryomyces* species possess greater halo-tolerance than most of the other genera, but the concn. of NaCl permitting optimum growth does not serve to differentiate between marine and terrestrial yeasts. (27 references.)
E. M. J.

Comparison of media for the enumeration of moulds and yeasts in foods and beverages. D. A. A. Mossel, M. Visser and W. H. J. Mengerink (*Lab. Pract., Lond.*, 1962, Feb., Reprint).—Comparative study of a simplified Moir and Russell glucose–yeast extract–agar (glucose 2% + dehydrated yeast extract 0·5%) (Mossel, 1951) and 'Mycophil' agar (Sharf, 1960) showed both to be equally favourable as a basal medium for yeasts and moulds, both at pH 6·6 and after acidification to pH 4·0 with tartaric acid. However, most *Lactobacillaceae* grow in both media at pH 4, while at lower pH values some moulds and yeasts are inhibited. Improved selectivity was obtained with glucose–yeast extract agar at pH 6·6 containing 100 μg. per ml. of oxytetracycline. In this medium bacterial growth is restricted to some Gram-negative rods (*Pseudomonas* and *Proteus* spp.). (29 references.) E. C. Apling.

Fungicidal activity of boric acid in the presence of various polyhydroxy-compounds. J. Ploquin and C. Ploquin (*Ann. pharm. franç.*, 1962, **20**, 201—210).—The effect of boric acid alone, polyhydroxy-compounds (including hydroxy-acids) alone and mixtures of the two on various fungi grown on Czapek–Dox medium, is studied. A synergistic effect is found in all cases. Account is taken of the presence of lactic acid in all the media. It is not yet possible to relate the synergistic effect with the effect of the polyhydroxy-compounds on the physical properties of boric acid solution.
E. J. H. Birch.

3.—SANITATION, WATER, etc.

Protection of food products. Société des Usines Chimiques Rhône-Poulenc (B.P. 875,352, 4.2.60. Fr., 6.2.59).—A food product is protected against fungal attack by forming thereon a film from an aq. emulsion of a polymer containing *N*-butylbenzamide (10—20 wt.-% on polymer). The preferred polymer is a vinyl polymer, e.g., polyvinyl acetate or a copolymer of vinyl acetate and vinyl chloride. F. R. Basford.

Purification of pyrethrum extract. Olin Mathieson Chemical Corp. (B.P. 875,760, 6.5.58. U.S., 13.5.57).—A crude conc. pyrethrum extract is purified by admixing a hydrocarbon solvent, b.p. 125—350° (e.g., kerosene); treating the mixture with $Na_3PO_4,12H_2O$ (0·01—1 wt.-% on mixture); heating for 1—24 h. at 40—70°; then cooling at −10° to −30° for 1—24 h.; and separating off the pptd. sludge. F. R. Basford.

Purification of warfarin sodium. Wisconsin Alumni Research Foundation (B.P. 874,149, 16.2.60. U.S., 25.2.29).—Aq. NaOH is added to a slurry of warfarin in water containing 10% of acetone by vol. and the solution clarified with C and spray-dried. The product is purified by dissolution in warm Pr^iOH (<0·5% water) and separated by cooling. It does not become discoloured on storage. F. R. Basford.

Water, wastes and sewage

Improved reagent for determining fluoride in potable water. C. K. Lim (*Analyst*, 1962, **87**, 197—201).—A solution of 0·7 g. of alizarin red S in 100 ml. of water is added to a solution of 0·45 g. of $ZrOCl_2,8H_2O$ in 100 ml. of water and 70 ml. of conc. H_2SO_4 added to 700 ml. of water and cooled is then added and the mixture is diluted to 1 litre and set aside for 1 h. To the sample of water and to a series of dilutions of a standard solution of NaF (1 ml. ≡ 0·05 mg. of F) the reagent is added and the colours are compared after 20 min. Al salts interfere and a table is provided indicating the correction to be added for the determined Al content. Differences of 0·05 p.p.m. can be determined in the range 0—1·5 p.p.m.
A. O. Jones.

Stream re-aeration. A. J. Wiley, B. F. Lueck, R. H. Scott and T. F. Wisniewski (*J. Wat. Pollut, Control Fed.*, 1962, **34**, 401—411).—Residual org. matter in streams may cause O_2 depletion to reach dangerously low levels, thus hindering the self-purification capacity of water-courses. Re-aeration has been successfully carried out by various methods including the use of weirs, cascades, mechanical diffusion and hydroturbine aeration. The last, where practicable, was considered to be the most successful, the dissolved O_2 level of a moving stream being increased by 2½ tons of O_2/day/1000 c.f.s. (11 references.) B. F. Fullan.

Phase-contrast microscopy for counting bacteria by direct method in studying quality of water. A. S. Razumov and L. E. Korsh (*Mikrobiologiya*, 1962, **31**, 357—361).—The standard method of counting bacteria of a group of enteric bacilli requires 24 h. and saprophyte bacilli 24—48 h., several times longer than that needed to purify water in a large water-works. By phase-contrast microscopy bacterial count can be made in 15—20 min. without dyeing organisms on filter. Water sample is passed through membrane filter which is then dried, placed on object glass in a drop of immersion oil and covered with thin cover glass (0·17 mm.). Detailed instructions for setting up microscope are given. Green illumination is used to reduce eyestrain. With practice bacteria can be distinguished from suspended particles from water. Close agreement was obtained between results of many determinations with the phase-contrast method and Razumov's earlier method in which bacteria on membrane filter are dyed with erythrosin.
P. W. B. Harrison.

Effect of humidity of air on viability of micro-organisms in aerosol. V. V. Vlodavets (*Mikrobiologiya*, 1962, **31**, 350—356).—Effect of air humidity on micro-organisms in drop phase of a bacterial aerosol was studied, using Gram-positive *Staphylococcus albus* and *Sarcina lutea* from laboratory air and Gram-negative *Escherichia coli* and *Bacterium prodigiosum* from water. Suspensions of each organism were dispersed in 250 l. chamber at 18·5—21°. R.H. was maintained at <20, 30—40, 50—60, 70—80 and >90%. Total bacteria in chamber were estimated by filtering sample through No. 4 membrane filter. Petri dishes with suitable medium for organism in use were exposed at moment of formation of aerosol, after 10 min. and after 20 min., for a period of 10 min. Table shows no. of colonies of *Staph. albus* developed at R.H. from 13 to 92%. Graphs show effect of R.H. on *B. coli* and *B. prodigiosum* for 10 and 20 min. at 5 different R.H. Fall in concn. of viable organisms depends on decay of organism at particular R.H. and on rate of sedimentation. *Staph. aureus* and *S. lutea* were relatively stable in water droplets in air with R.H. 12—98%. Removal was mainly due to sedimentation. *E. coli* and *B. prodigiosum* were much less stable in aerosol and died rapidly below R.H. 50%. Above 70% their viability was least affected. (20 references.)
P. W. B. Harrison.

Interstage elutriation of digested sludge. A. H. Chasick and R. T. Dewling (*J. Wat. Pollut. Control Fed.*, 1962, **34**, 390—400).—When sludges averaged less than 4% solids, elutriation was necessary in a secondary digestion tank to increase the sludge concn. and thus reduce its vol. Elutriation was carried out in a 103-ft., 4 in. dia. flat-bottomed digestion tank and was compatible with normal operating procedure. Plant layouts and operating snags were discussed. B. F. Fullan.

4.—APPARATUS AND UNCLASSIFIED

Paper-chromatographic determination of ascorbic acid, dehydroascorbic acid and ascorbigen in heated biological material. III. Paper-chromatographic determination of ascorbigen. J. Herrmann and M. Zobel (*Z. LebensmittUntersuch.*, 1962, **117**, 189—202).—It is shown that ascorbigen remains stable under the conditions previously described (cf. *ibid.*, 1962, **116**, 477, **117**, 1) for the extraction of the accompanying substances (by aq. 2% oxalic acid) and their determination. The ascorbigen is quant. hydrolysed to ascorbic acid (**I**) by heating an aliquot of the acid extract (>5 h. old) in boiling water during 10 min.; this is accomplished in a closed ampoule after having expelled the air by CO_2. The **I** remains stable during the hydrolysis. The difference between the amounts of **I** found after and without hydrolysis gives a measure of the ascorbigen. In comparison with the polarographic and the photometric method, this method shows greater reproducibility of results and wider possibilities of application. Results for **I**, dehydroascorbic acid and ascorbigen are given for seven specimens of biological material. (45 references.) P. S. Arup.

Method for separating lipid components of leaves. V. H. Booth (*Biochem. J.*, 1962, **84**, 444—448).—Green leaves are extracted with $COMe_2$ and the extracted material, without being hydrolysed or subjected to column chromatography, is transferred to light petroleum and chromatographed on paper in two dimensions. The chromatograms are developed (ascending) with 1% of $COMe_2$–99% light petroleum (b.p. 40—60°), and then second dimension chromatograms are obtained by development (ascending) with MeOH–water (23 : 2, v/v). The method, which is much simpler than others, is sensitive to low concn. of many lipid substances and many phyllolipids are separated in less than 5 h. on one chromatogram. (23 references.) J. N. Ashley.

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