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ASSESSMENT OF MEAT FRESHNESS IN QUALITY CONTROL EMPLOYING CHEMICAL TECHNIQUES: A REVIEW

By D. PEARSON

Numerous chemical and physical tests have been described which reflect the biochemical and other changes which occur in meat during storage. Although some work has occasionally included the correlation with organoleptic data, there have been few suggestions as to possible critical legal or control limits. In industrial control, however, each method has to be considered in relation to the purpose intended and semi-empirical tests may be of value provided that the procedure is standardised. Critical maxima based on chemical values have been recommended for white fish, which differs, however, from meat in having a negligible fat content. With meat, protein breakdown usually precedes fat spoilage, but in certain circumstances the order of these reactions is reversed. Recommended limits for meat should take into account both types of spoilage. The review concludes with suggestions for a co-ordinated programme of study which could help in clarifying some of the difficulties inherent in the application of meat spoilage research in industrial control.

Introduction

From the point of view of public health inspection and industrial control it would be desirable if critical limits of acceptability could be applied with reasonable confidence to meat. As the exact limits applied are liable to be varied according to various factors and circumstances, it is equally desirable to have available suitable standardised methods for spoilage assessment.

Microbiological methods, although most desirable from the theoretical viewpoint, are usually too lengthy for efficient industrial control. Numerous relatively rapid chemical methods might be considered, however, for measuring the limits of acceptability for meat. Because of the influence of the fat content, the study of meat in this connexion is more complex than with white fish for which various maximum limits have been recommended.[1]

The main practical operations involved in producing meat for the retail or industrial consumer are as follows:

- Animal care and feeding.
- Pre-slaughter care of the animal.
- Slaughterhouse operations, e.g. stunning, pithing, bleeding, de-hairing, dressing.
- Conditioning.
- Storage of the carcass.
- Cutting, mincing, etc.

The quality of the final product is markedly affected by the care taken at each of these stages. In view of this, animals are preferably rested prior to slaughter to give maximum lactic acid production. In turn hygiene precautions are taken in the slaughterhouse to reduce microbial contamination. *Post-mortem* the loss of ability to re-synthesise adenosine triphosphate below pH 5·5 is a major consequence of circulatory failure.[2] The lack of oxygen causes a fall in redox potential. Owing to the reduced water binding capacity near the iso-electric point, coupled with protein denaturation, fluid exudation occurs at about pH 5. Also the colour becomes browner due to oxidation of myoglobin to met-myoglobin.

The healthy animal can normally cope with bacterial invasion, but in meat the fall in redox potential encourages the growth of anaerobes.[3] Also the accumulation of metabolites arising from breakdown of nucleotides, carbohydrates and proteins presents a good culture medium for bacteria. The keeping qualities may be further affected if the meat has been frozen and thawed out before mincing, etc.

Criteria to be considered for methods suitable for assessing the spoilage of meat

A test for assessing spoilage in industrial control should if possible meet the following criteria:

- It should be relatively rapid in performance.
- There should be reasonably good agreement between replicate determinations on the same sample or extracts obtained from it.
- Where appropriate, it should give good recoveries.
- It should give reasonably consistent values at the fresh and spoiling stages, so that critical values can be applied.
- It should be reasonably sensitive in the incipient stages of spoilage.
- The values should rise or fall progressively as spoilage advances, so that recommended maximum or minimum values for the critical stage between fresh and spoiled can be stated with reasonable confidence.
- It should be correlated reasonably well with organoleptic findings, particularly as regards distinguishing between fresh, spoiling and spoiled meat.

In the application of any method the importance of good sampling of meats cannot, of course, be over-emphasised.

Possible methods for assessing the spoilage of meat

Various papers in the literature discuss selected biochemical changes that take place in meat during spoilage. Apart from the effect of pH very few workers have attempted to suggest tentative limits of acceptability. In fact Jensen[4] and Turner[5] infer that chemical methods are unlikely to be applicable for

specification purposes. As some success has been achieved in this direction with the somewhat comparable but admittedly simpler material, white fish,[1,6–9] the problem would appear to deserve further attention. Further, some of the methods used for fish do not appear to have been applied to meat.

The published methods which relate to the changes which take place during the storage of meat and fish can be conveniently classified as follows:

Methods related to spoilage of lean meat and in particular to the breakdown of protein.
Methods related to fat spoilage.
Miscellaneous techniques including some which measure physical changes.
Microbiological methods.
Organoleptic methods.

Chemical methods of assessment relating to protein breakdown

Owing to the wide variation in fat content between different cuts, the protein content of meat may vary from about 1 to 20%. The first stages of protein breakdown due to the proteolytic enzymes of bacteria are probably similar to those which occur when mineral acids are added, so that soluble peptones and later amino acids are formed. The actual reactions which take place vary, however, according to the bacterium, the temperature and whether the conditions are aerobic or anaerobic. Gardner[10] confirmed the findings of Ayres[11,12] that the psychrophilic bacteria growing on and causing spoilage in beef were mostly *Pseudomonas*, which are likely to cause the production of ammonia by de-amination of amino acids under aerobic conditions. Unlike the situation with fish, trimethylamine is not produced to a significant extent in meat.[13]

Methods involving the determination of volatile bases

The volatile bases in most species of fish consist of ammonia together with appreciable quantities of amines. In meat trimethylamine is only present in insignificant quantities and the volatile nitrogen (TVN) consists almost entirely of ammonia.[13] As ammonia production due to de-amination of protein increases during spoilage, its determination represents a simple method of following the course of deterioration of the lean meat. Most of the methods used for determining the volatile bases in fish and meat involve macro or semi-micro distillation, micro-diffusion, aeration or colorimetry. The first three groups involve treating the minced material or an extract of it with a relatively weak alkali, and the volatile base is distilled or diffused over into standard acid. Richardson & Scherubel[14] distilled the 'ammoniacal nitrogen' over in the presence of magnesium oxide. On addition of further water and repetition of the distillation, however, more base came over possibly because of the effect of heat on the protein. In consequence the procedure was standardised as to distillate volume and time of distillation. In view of the possible interference from such side reactions it is preferable to employ essentially the shorter distillation technique proposed for fish by Lucke & Geidel.[15] There are also advantages in absorbing the volatile nitrogen in boric acid rather than in standard acid.[16] Using this technique Pearson[17] found that minced beef sold in retail establishments gave TVN figures ranging from 10·6 to 19·3 mgN/100 g. A maximum acceptability limit of 20 mg volatile for N fat-free meat (TVN/FF) was later recommended for beef.[18] Although figures for ammoniacal nitrogen are reported by Richardson & Scherubel,[14] these authors appear to have used lean meat with fat contents below 5%, so that the values are not necessarily comparable with those obtained with fatter cuts. Turner[5] using de-proteinated extracts for the distillation found that the method was unsuitable for detecting incipient spoilage. There may be some advantages in employing vacuum distillation at lower temperatures as this prevents 'extra' breakdown of protein, and only volatile base present when sampled is determined, irrespective of time of heating.[19]

There are no reports in the literature of the application to meat of the semi-micro distillation procedure of Ronold & Jakobsen,[20] in which the volatile bases are steam-distilled from an alcoholic extract of fish using barium hydroxide. Similarly the Conway technique which has been extensively used for fish[21] does not appear to have been applied to meat except for checking the results of other methods (Burks *et al*[13]). For routine industrial control, diffusion overnight at room temperature could be replaced by a shorter procedure at a higher temperature. Spinelli[22] has stressed the importance of taking great care at each stage of the Conway method in order to obtain consistent results. Further, Wittfogel[23] showed that micro-diffusion has no particular advantage over the classical distillation method.

In the aeration method which is usually attributed to Folin, a meat macerate is mixed with alkali and aerated so that the ammonia is absorbed in acid for a standardised period before titration. Using such a technique, Falk & McGuire[24] reported ammonia contents of 3–10 mg nitrogen per 100 g in fresh beef. On storage the meat was not necessarily unpalatable until the value reached at least 30 mg. One disadvantage of the aeration method is that it may take several hours for the ammonia to be released. Falk[25] appears to prefer the method of Folin & Bell[26] in which the ammonia is extracted from a meat filtrate with permutit. The ammonia is then liberated by treatment with alkali and determined colorimetrically with Nessler's reagent. This method could be promising for control purposes if the time for preparation of the extract could be shortened.

Other methods related to protein breakdown

The determination of amino nitrogen has been used by some workers in the meat field. Determination by means of the Van Slyke method and the formol titration method is described officially in the U.S.A.[27] Using the formol titration, Broumand, Ball & Stier[28] stated that although the free amino nitrogen of lean samples showed a tendency to increase, this was not always pronounced or consistent. The 'ninhydrin-positive substances' (alpha-amino acids) as determined by Jay[29] and Saffle, May, Hamid & Irby[30] tend to show only slight changes as spoilage progresses. Similarly the reviews of Winton & Winton[31] and Turner[5] stress that determinations of indole and hydrogen sulphide only show significant increases over the fresh material when spoilage is in the advanced stages.

The degree of autolytic and bacterial proteolysis has been assessed in fish by means of the tyrosine value, which was originally developed by Bradley & Bailey.[32] Although the method cannot be considered to be specific for proteolysis, the tyrosine value has been regarded by Canadian workers, in particular, as affording a good general index of protein breakdown in fish, including herrings.[33] Therefore the technique could prove useful for the assessment of spoilage in

meat, particularly if a more rapid modification is employed.[34]

The amount of buffer-extractable nitrogen decreases during frozen storage, whereas the products of proteolysis increase. Above freezing, however, whilst protein breakdown is considerable, the changes in extractability of the nitrogen fraction or in the available -SH groups are negligible. Khan[35] therefore proposed, for assessing deterioration in poultry, the use of the 'quality index', which represents the ratio of the SH groups to the products of protein breakdown (tyrosine value). The application of the method to red meats would appear to be worthy of investigation.

Chemical methods of assessment relating to fat spoilage

The fat content of meat varies from 5% for lean tissue to over 90%. The fat of adipose tissue consists almost entirely of true fat or triglyceride. The main fatty acids present in meat fats are shown in Table I.

TABLE I

Main fatty acids in meat fats[36]

Fatty acid	Beef fat mean %	Mutton fat mean %	Pork fat mean %
Oleic acid	42	39	46
Palmitic acid	29	25	28
Stearic acid	20	25	13
Linoleic acid	2	4	10

In consequence, beef and mutton fats in particular have a low iodine value (approx. 45) and high melting point. Pork fat has a higher iodine value of about 65, but with pigs which have been fed on cake containing a highly unsaturated oil the figure for the resulting lard may be considerably higher. Apart from the triglycerides, animal fats contain small proportions of phospholipids, sterols, carotenoid pigments and fat-soluble vitamins. Many of the components may be altered during spoilage and numerous methods are available which are related to such changes:

Methods which measure the free fatty acids due to lipase action on the triglycerides.

Methods which measure oxidative rancidity due to the action of the air and ketonic rancidity due to micro-organisms.

Such types of deterioration affect the odour and taste and thereby limit the storage life of the meat. As fat rancidity reactions are accelerated by heat, it is preferable to determine the spoilage values on a chloroform extract obtained by cold maceration.[37,38]

Free fatty acids (FFA)

Various methods for determining the FFA in oils and fats are included in the review of Mehlenbacher.[39] The fatty acids present, being weak acids, can be titrated with strong caustic alkali. As fats and fatty acids are insoluble in water the titration is usually carried out in organic solvents such as ethyl alcohol,[40] alcohol–diethyl ether,[41] alcohol–chloroform, benzene, alcohol–benzene, isopropanol and isopropanol–toluene.

There are few figures quoted in the literature relating to the acidity of the fat of stored beef. Lea[42] reported figures of 1·5% (as oleic acid) in the fat of beef stored for 25 days at 0°. After this the acidity increased more rapidly in such a way that figures from 5 to 11% were found after 42 days, and this coincided with a change in the flavour of cooked joints from sweet to unpleasant. Although no critical limit is suggested, examination of Lea's paper gives the impression that flavour deterioration in beef and mutton was noticeable when the acidity of the extracted fat reached 2–3% (calculated as oleic acid). Samples of lamb and beef examined by Broumand, Ball & Stier[28] showed a progressive increase in the FFA figure during storage. White[43] suggested, however, that spoilage in bacon fat due to excessive acidity is normally of little importance. Pearson[17] reported that minced beef sold in retail establishments gave figures ranging from 0·38 to 1·74% FFA (calculated as oleic acid on the extracted fat). In view of the progressive rise in the FFA figure during storage a maximum acceptability limit of 1·8% was recommended.[18]

Peroxide value

Theories relating to the autoxidation of unsaturated fatty acids postulate the primary formation of substances possessing peroxide properties. Although the peroxides can be measured from the oxidation of titanous or ferrous salts, the most frequently used methods depend on the reaction of alkali iodide in acid solution with the bound oxygen followed by titration of the liberated iodine with sodium thiosulphate.[39] The result is calculated as milliequivalents of peroxide per kg of sample (ml N thiosulphate/kg) or as ml of N/500 thiosulphate per g. Most workers have employed acetic acid–chloroform as solvent and reduced side effects due to air by boiling the mixture or by passing inert gas through it. Lea[44] showed that different results were obtained according to the procedure employed and attributed this to the non-uniform reactivity of the peroxides and to differing degrees of re-absorption of the liberated iodine at the positions of unsaturation. By keeping the mixture boiling throughout under a long reflux condenser, interference from oxygen is reduced to a minimum.[45] Colorimetric procedures for determining peroxide values in oils and fats have also been described.[46–48]

Most workers who have reported results from the application of peroxide value determinations to the fat extracted from stored meat have found, as with 'pure oils', an induction period followed by a fairly sharp rise.[28,49] With bacon fat, White[43] stated that: 'None of the coefficients between peroxide oxygen and the physical and the chemical characteristics of the fat considered to be related to the development of rancidity reached the level of statistical significance'. Zipser, Kwon & Watts[50] have stressed the difficulties in designating a threshold peroxide value for rancid odour with both cured and uncured meats.

Thiobarbituric acid number or value (TBA)

Several workers have suggested that the TBA method can be used as an empirical measure of the deterioration of fatty foods. Kohn & Liversedge[51] reported that a red colour was produced when tissue was incubated with 2-thiobarbituric acid. Work on rancid milk fat[52] and salmon oil[53] suggested from spectrum and chromatograph measurements that malonaldehyde was involved in the TBA reaction. In recent years therefore the TBA values have been recorded in terms of malonaldehyde rather than merely as the optical density.

The results obtained in the TBA test vary according to the conditions laid down, and all the methods described in the literature are essentially empirical in nature. As non-extract-

able material may react with TBA it is preferable to apply the test to the intact beef sample, or a distillate of it, rather than to the extracted fat. Earlier, workers heated a slurry of the flesh with the TBA reagent[54,55] and measured the optical density of the pigment after extraction with amyl alcohol–pyridine mixture. More recently many of the papers in the literature[50,56,57] have employed the technique described by Tarladgis, Watts, Younathan & Dugan[58] in which the TBA reaction is applied to a distillate obtained from the acidified meat. In this method the result is calculated as malonaldehyde on the assumption that the recovery figure of the method is 68%. The interference in the reaction due to degradation products[59] has been overcome by avoiding the use of acid in the final reaction.[60]

Unlike other methods related to oxidative spoilage, the TBA test has the advantage that it can measure the deterioration in both extractable and non-extractable lipids, and comparatively high TBA levels have been reported in heavily pigmented beef.[56] The high TBA numbers found in lean beef (compared with pork) are probably due to the comparatively high phospholipid content. It is apparent that, whereas peroxides are intermediates in the oxidative decomposition of unsaturated fatty acids, malonaldehyde is an end product.[50] The correlation between odour and TBA numbers is high and there may be advantages in considering peroxide/TBA ratios rather than single values.[50] As the TBA test, as applied to the intact sample, appears to reflect the condition of the lean tissue it may be useful for general spoilage assessments, provided it gives reasonably consistent values at the fresh and spoiling stages.

Ranco number

Ramsey, Kemp & Grainger[61] have developed a method in which rendered pork fat is heated at 70° with a solution of potassium hydroxide in isopropyl alcohol under standardised conditions. Rancid fats produce a yellow colour, and the optical density measured at 385 nm is termed the 'Ranco number'; this showed a very high correlation with TBA values. The method, however, was applied to spoiled rendered fat and not to that obtained from the meat itself at various stages of storage.

Kreis test

The various methods which have been proposed for carrying out the Kreis test, which is based on the reaction between phloroglucinol and the fat under acidic conditions, have been reviewed by Mehlenbacher.[29] The red colour produced appears to be related to oxygen absorption and may be due to the presence of epihydrin aldehyde[62] or malonaldehyde.[52] The colour produced can be assessed visually[63] or absorptiometrically.[43,64]

There are very few references to the application of the Kreis test to fat extracted from stored meat. The general opinion of the method is that it is too sensitive in the incipient stages so that non-rancid fats sometimes produce intense colours. Pool & Prater[64] however, have reported two figures for pork fat (rendered) based on their method, viz. transmission (%) 69·0 and 36·3. White[43] noted that the Kreis test is the most sensitive of the rancidity methods which he applied to bacon fat.

Estimation of carbonyl compounds

Attempts have been made to determine the total carbonylic substances formed by secondary degradation of the first-formed peroxides. The most widely used method is probably the colorimetric procedure of Henick, Benca & Mitchell,[65] but the benzidine test of Holm, Ekbom & Wode[66] has shown some correlation with off-flavour development. Although it is probable that volatile carbonylic degradation products from oxidising fats are important in relation to odour, their determination appears to be difficult as they seem to be formed during their estimation.

Ketone or perfume rancidity

Ketone rancidity is probably caused by moulds and is encouraged by the presence of moisture and protein. Perfume rancidity can be detected by the method of Taufel & Thaler[67] in which salicylaldehyde reacts with the ketones in acid solution to form red condensation products. The method appears to have been applied largely to butter and margarine.

Iodine value

Although fats become more saturated as they go rancid the variation in the iodine value of the extracted material appears to be too small and irregular to render it suitable for spoilage assessments.[68]

Miscellaneous and general techniques for spoilage assessment

Certain methods which might be considered for the assessment of spoilage are associated with chemical and physical changes which take place in stored meat. They are not necessarily directly associated with the deterioration of protein or fat.

pH value

Barnes & Ingram[3] have described a method for the determination of the pH of meat muscle using a glass electrode in which the sample is chopped up in sodium iodoacetate solution to inactivate enzymes. Industrially, spear electrodes are pushed into the meat, and the reading is taken as soon as the needle on the pH meter settles to a constant value.

Bodwell, Pearson & Spooner[69] reported that the average initial pH value for five beef carcasses of 6·99 declined to 5·46 and 5·57 in 48 and 480 hours respectively. Such findings of a decline followed by a rise in the pH of meat examined from the slaughter stage have been reported by various workers.[3,70,71] Van Logtestijn[72] includes the following among criteria to be used as a basis for the evaluation of the keeping quality of meat (provided the pH is determined at least 24 hours after slaughter to allow for fall during *rigor*): meat with a final pH above 6·5 should only be used for the manufacture of heat-treated products; meat with a pH value between 6·0 and 6·5 should be submitted to a further examination.

The practical advantage of using the pH determination lies in its simplicity and rapidity.

Total acidity

The direct titration of the total acidity of meat has also been reported.[14,28] Although the lactic acid has an important influence on the taste, the total acidity only increases slightly during storage.

Volatile acidity

Methods developed for determining the volatile acidity of

fish[27] do not appear to have been applied to any extent to meat. The lactic acid content appears to reach a constant level within 1–2 days of slaughter.[69] Acetic, butyric and propionic acids have been shown to predominate in the volatile acids from the beef carcasses examined by Shank, Silliker & Goeser,[73] who suggest that they are of importance in the development of a sour non-microbial off-condition.

Oxidation–reduction potential

Oxidation–reduction potential is usually measured by means of platinum and calomel electrodes after adjusting the pH meter with quinhydrone buffer solutions. Barnes & Ingram[3] found that the potential in horse muscle after death fell from +250 mV to −130 mV. Owing to the withdrawal of the last traces of oxygen from the tissue the greatest change occurs immediately after death. If bacterial growth occurs, the potential may fall below −250 mV. It is usually considered difficult to measure redox potential with certainty and figures quoted are essentially comparative.

Reductase tests using dyes

The principle involved in reductase tests is that certain dyes can act as artificial acceptors for the hydrogen, which is transported during the metabolism by dehydrogenases. The dyes are chosen so that they show colour changes over the redox potential range associated with spoilage. A few promising results have been reported with meats by measuring the times required to reduce methylene blue[71,74] and reasuzurin.[75]

Reducing substances

The volatile reducing substances (VRS) have been determined principally on fish.[76]. From the pressed-out juice the VRS is aerated into alkaline permanganate and the extent of reduction is measured by titration.[77] The highest VRS figure for meat with a 'foul, putrid odour' quoted by Lang *et al.*[76] seems to correspond to a comparatively small titration difference, so the method appears to lack sensitivity for assessing incipient spoilage. The direct titration of the total reducing substances with iodine has been applied to fish by Truttwin.[78]

Water-holding capacity

The water-holding capacity (w.h.c.), i.e. the ability of meat to hold fast to its own or added water during the application of pressure or mincing, appears to be influenced by the treatment the animal receives prior to slaughter. Although the mechanism of the protein hydration is not well understood, there is much evidence that the changes in the w.h.c. of meat are a sensitive indicator of alterations in the proteins.[79] The w.h.c. of freshly slaughtered meat is high, but it drops markedly within a few hours and then increases slowly during further storage. Hamm[80] attributed two-thirds of the *post-mortem* hydration drop to the breakdown of the ATP and one-third to the fall in pH, so that there is probably a connexion between the hydration decrease and *rigor* development.

As the results obtained of measurements of the w.h.c. vary according to the technique employed, they can only reflect the proportion of immobilised water on a comparative basis. Grau & Hamm[81] and Wierbicki & Doatherage[82] calculate the area of water which diffuses from the meat on to a filter paper under the influence of a standardised but manually applied pressure. Jay[83] found that during spoilage the free water area decreased linearly with time, and the fall was closely related to the increase in bacterial numbers. Also meat infused with chlortetracycline showed a lower bacterial count and higher w.h.c. when compared with control meat. This suggests that the increased w.h.c. accompanying incipient spoilage is the result of bacterial proteolysis, during which the splitting of the peptide bonds results in a loosening of protein structure so that more water-binding sites are available.

Extract release volume (e.r.v.)

Jay[70] has developed a method for determining the extract release volume (e.r.v.), which is related to the water-holding capacity and appears to have considerable possibilities for assessing the spoilage of beef. The procedure is based on measuring the volume of aqueous filtrate released from a slurry of meat in a fixed time. The e.r.v. decreases as spoilage progresses and no filtrate at all is obtained with putrid meat. After standardising the procedure using 25 g meat with 100 ml buffer solution (pH 5·8) and a filtration time of 15 min, Jay[29] found that only 3 of the 40 samples examined gave filtrate volumes below 30 ml. After considering the correlation with several organoleptic factors, Jay & Kontou[84] suggested than an e.r.v. of 25 ml was more appropriate as a rejection cut-off figure. One drawback is the fairly wide range of values given by fresh meats (21–35 ml). Using 15 g beef with 60 ml buffer solution an e.r.v. minimum figure of 20 ml for acceptability has also been proposed.[18] Jay[85] found that the correlation between e.r.v. and w.h.c. was highly significant.

In view of its simplicity, rapidity in performance and the apparently consistent decrease during spoilage, the e.r.v. may well prove useful for routine control assessments of meat quality.

Hypoxanthine

Various species of fish have been found to contain increasing amounts of hypoxanthine as spoilage in ice increases.[86,87] Work on meat, however, suggests that the method would show a wide variation for the fresh baseline values.[88]

Volatile compounds

In general the action of bacteria upon meat constituents produces the stale, sour or putrid odours associated with spoilage. Stahl[89] condensed the volatiles from frozen beef at low temperatures and separated the volatiles in the various fractions by g.l.c. Using mass spectrometry the eluted odorous compounds were identified as hydrogen sulphide and methyl and ethyl mercaptans. By employing a somewhat similar technique, Merritt and co-workers[90] reported that acetaldehyde, acetone, methyl ethyl ketone, methanol and ethanol were also present. Burks and his colleagues[13] also employing g.l.c., found that the volatiles from raw beef consisted of 99·9% ammonia. Hornstein & Crowe[91] have referred to the isolation of volatile carbonyl compounds and free fatty acids (after conversion to the esters) from meat fats. There is considerable evidence that volatiles from lean meat contribute to the flavour and that species flavour differences can be traced to the fat.

Conclusion

Although much unpublished work may have been performed by industrial organisations a study of the literature reveals that there have been relatively few attempts to apply the results of biochemical findings on stored meat to control work. In general it has usually been assumed that as the spoilage pattern in meat is very complex it is not possible to apply numerical limits of acceptability. With few exceptions there is a paucity of data on the range of values given by fresh meat, but such information is all important in deciding whether any particular analytical method is suitable for industrial control. It is equally necessary to ascertain by correlation with organoleptic considerations whether distinctive values are obtained at the critical spoiling stage.

During spoilage the products of protein breakdown tend to affect the organoleptic characteristics before fat deterioration is apparent. In consequence, correlated work starting with beef and covering determinations of de-amination products such as ammonia and Khan's quality index should form a useful field of study. Consideration must also be given to fat hydrolysis and rancidity, which are occasionally the predominating factors during storage. In spite of the long-standing use of the peroxide value for reflecting oxidative changes, difficulties in interpretation suggest that the thiobarbituric acid value might be a more useful guide, particularly as it reveals changes in the lean as well as the adipose tissue. Combination of all results with determinations of pH values and extract-release volumes, together with studies of the gas chromatograms of the volatiles should then show which of the various tests is most likely to be suitable for control work. There would then be a need for official and industrial laboratories to apply the recommended methods to meat received and seek guidance from experienced inspectors as to the suitability of the proposed limits. In the meantime such correlations could be applied immediately to the relatively simple but promising determinations of TVN, FFA and e.r.v.

National College of Food Technology,
(University of Reading),
St. George's Avenue,
Weybridge, Surrey

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References

1. Tomiyasu, Y., & Zenitani, B., *Adv. Fd Res.*, 1957, **7,** 41
2. Lawrie, R. A., 'Recent advances in food science' (Edited by Hawthorn, J., & Leitch, J. M.), 1962, **1,** 68 (London: Butterworths)
3. Barnes, E. M., & Ingram, M., *J. Sci. Fd Agric.*, 1955, **6,** 448
4. Jensen, L. B., 'Microbiology of meats', 1954, 3rd Edn., p. 306 (Illinois, U.S.A.: Garrard Press)
5. Turner, A., *Fd Mf.*, 1960, **35,** 386
6, 7. Ehrenberg, A. S. C., & Shewan, J. M., *J. Sci. Fd Agric.*, 1953, **4,** 482; 1955, **6,** 207
8. Shewan, J. M., Macintosh, R. G., Tucker, C. G., & Ehrenberg, A. S. C., *ibid.*, 1953, **4,** 283
9. Shewan, J. M., & Ehrenberg, A. S. C., *ibid.*, 1957, **8,** 227
10. Gardner, G. A., *J. appl. Bact.*, 1965, **28,** 252
11. Ayres, J. C., *Fd Res.*, 1960, **25,** 1
12. Ayres, J. C., *J. appl. Bact.*, 1960, **23,** 471
13. Burks, R. E., Baker, E. B., Clark, P., Esslinger, J., & Lacey, J. C., *J. agric. Fd Chem.*, 1959, **7,** 778
14. Richardson, W. D., & Scherubel, E., *J. Am. chem. Soc.*, 1908, **30,** 1515
15. Lucke, F., & Geidel, W., *Z. Unters. Lebensmittel*, 1935, **70,** 441
16. Pearson, D., 'Chemical analysis of foods', 1962, 5th Edn., p. 309 (London: Churchill)
17. Pearson, D., *J. Ass. publ. Analysts*, 1966, **4,** 90
18. Pearson, D., *Fd Mf.*, 1967, **42** (11), 42
19. Tomiyama, T., da Costa, A. A., & Stern, J. A., *Fd Technol., Champaign*, 1956, **10,** 614
20. Ronold, O. A., & Jakobsen, F., *J. Soc. chem. Ind. Lond.*, 1947, **66,** 160
21. Pearson, D., *Lab. Pract.*, 1962, **11,** 538
22. Spinelli, J., *Fishery Ind. Res.*, 1964 **2** (2), 17
23. Wittfogel, H., *Archiv. Lebensmittlehyg.*, 1960, **11** (4), 84
24. Falk, K. G., & McGuire, G., *J. biol. Chem.*, 1919, **37,** 547
25. Falk, K. G., *J. Ass. off. agric. Chem.*, 1924, **8,** 160
26. Folin, O., & Bell, R. D., *J. biol. Chem.*, 1917, **29,** 329
27. Official Methods of Analysis of the Association of Official Agricultural Chemists, 1945 and 1965, 6th and 10th Edn. (Washington, D.C., U.S.A., Association of Official Agricultural Chemists)
28. Broumand, H., Ball, C. O., & Stier, E. F., *Fd Technol., Champaign*, 1958, **12,** 159
29. Jay, J. M., *Fd Technol., Champaign*, 1964, **18** (10), 133
30. Saffle, R. L., May, K. N., Hamid, H. A., & Irby, J. D., *ibid.*, 1961, **15,** 465
31. Winton, A. L., & Winton, K. B., 'The structure and composition of foods', 1949, **3,** p. 336–343 (New York: Wiley; London: Chapman and Hall)
32. Bradley, H. C., & Bailey, B. E., *Fd Res.*, 1940, **5,** 487
33. Sigurdsson, G. J., *Ind. Engng Chem. analyt. Edn.*, 1947, **19,** 892
34. Wood, A. J., Sigurdsson, G. J., & Dyer, W. J., *J. Fish. Res. Bd Can.*, 1942, **6,** 53
35. Khan, A. W., *Nature, Lond.*, 1965, **208,** 204
36. Lea, C. H., 'Recent advances in food science' (Edited by Hawthorn, J., & Leitch, J. M.), 1962, **1,** 83 (London: Butterworths)
37. Rockwood, B. N., Ramsbottom, J. M., & Mehlenbacher, V. C., *Ind. Engng Chem. analyt. Edn.*, 1947, **19,** 853
38. Pearson, D., *J. Ass. publ. Analysts*, 1965, **3,** 76
39. Mehlenbacher, V. C., 'The analysis of fats and oils', 1960 (Illinois, U.S.A.: Garrard Press)
40. British Standards Institution, Methods of analysis of oils and fats, B.S. 684: 1958 (London: B.S.I.)
41. British Pharmacopoeia, 1963, p. 1055 (London: General Medical Council)
42. Lea, C. H., *J. Soc. chem. Ind., Lond.*, 1931, **50,** 215 T
43. White, W. H., *Can. J. Res.*, 1941, **19** (D), 278
44. Lea, C. H., 'Rancidity in edible fats', 1938, Special Report of the Food Investigation Board No. 46 (London: H.M.S.O.)
45. Sully, B. D., *Analyst, Lond.*, 1954, **79,** 86
46. Hills, G., Loftus & Thiel, C. C., *J. Dairy Res.*, 1946, **14,** 340
47. Driver, M. G., Koch, R. B., & Salwin, H., *J. Am. Oil Chem. Soc.*, 1963, **40,** 504
48. Swoboda, P. A. T., & Lea, C. H., *Chemy Ind.*, 1958, No. 33, 1090
49. Watts, B. M., 'Lipids and their oxidation', 1962 (Editor Schultz, H. W.) p. 202 (Conn., U.S.A., Avi)
50. Zipser, M. W., Kwon, T-W., & Watts, B. M., *J. agric. Fd Chem.*, 1964, **12,** 105
51. Kohn, H. I., & Liversedge, M., *J. Pharmac. exp. Ther.*, 1944, **82,** 292
52. Patton, S., Keeney, M., & Kurtz, G. W., *J. Am. Oil Chem. Soc.*, 1951, **28,** 391
53. Sinnhuber, R. O., Yu, T. C., & Yu, Te C., *Fd Res.*, 1958, **23,** 626
54. Turner, E. W., Paynter, W. D., Montie, E. J., Besser, M. W., Struck, G. M., & Olson, F. C., *Fd Technol., Champaign*, 1954, **8,** 326
55. Schwartz, M. G., & Watts, B. M., *Fd Res.*, 1957, **22** (1), 76
56. Keskinel, A., Ayres, J. C., & Snyder, H. E., *Fd Technol., Champaign*, 1964, **18** (2), 101
57. Zipser, M. W., & Watts, B. M., *ibid.*, 1961, **15,** 445
58. Tarladgis, B. G., Watts, B. M., Younathan, M. T., & Dugan, L., *J. Am. Oil Chem. Soc.*, 1960, **37,** 44
59. Tarladgis, B. G., Pearson, A. M., & Dugan, L. R., *J. Am. Oil Chem. Soc.*, 1962, **39,** 34
60. Tarladgis, B. G., Pearson, A. M., & Dugan, L. R., *J. Sci. Fd Agric.*, 1964, **15,** 602
61. Ramsey, C. B., Kemp, J. D., & Grainger, R. B., *Fd Technol., Champaign*, 1964, **18** (1), 105
62. Powick, W. C., *J. agric. Res.*, 1923, **26,** 323
63. Walters, W. P., Muers, M. M., & Anderson, E. B., *J. Soc. chem. Ind., Lond.*, 1938, **57,** 53 T

64. Pool, M. F., & Prater, A. N., *Oil Soap*, 1945, **22,** 215
65. Henick, A. S., Benca, M. F., & Mitchell, J. H., *J. Am. Oil Chem. Soc.*, 1954, **31,** 88
66. Holm, U., Ekbom, K., & Wode, G., *ibid.*, 1957, **34,** 606
67. Taufel, von K., & Thaler, H., *Chemikerzeitung*, 1932, **56,** 265
68. Pearson, D., Unpublished
69. Bodwell, C. E., Pearson, A. M., & Spooner, M. E., *J. Fd Sci.*, 1965, **30,** 766
70. Jay, J. M., *Fd Technol., Champaign*, 1964, **18** (10), 129
71. Rogers, R. E., & McCleskey, C. S., *ibid.*, 1961, **15** (4), 210
72. Logtestijn, J. G., *Fleischwirtschaft*, 1965, **45,** 916
73. Shank, J. L., Silliker, J. H., & Goeser, P. A., *Appl. Microbiol.*, 1962, **10,** 240
74. Rogers, R. E., Brown, L. R., & McCleskey, C. S., *Bact. Proc.*, 1958, A63, 25
75. Wilhelmsen, J. B., 'Some quick methods for the quality control of meats', 1965, Manuskript nr. 306 E (Roskilde, Denmark: Danish Meat Research Institute)
76. Lang, O. W., Farber, L., Beck, C., & Yerman, F., *Ind. Engng Chem. analyt. Edn.*, 1944, **16,** 490
77. Farber, L., & Ferro, M., *Fd Technol. Champaign*, 1956, **10,** 303
78. Truttwin, H., *Z. Unters. Lebensmittel*, 1953, **97,** 275
79. Hamm, R., *Adv. Fd Res.*, 1960, **10,** 355
80. Hamm, R., *Biochem. Z.*, 1956, **328,** 309
81. Grau, R., & Hamm, R., *Naturwissenschaften*, 1953, **40,** 29
82. Wierbicki, E., & Deatherage, F. E., *J. agric. Fd Chem.*, 1958, **6,** 387
83. Jay, J. M., *Appl. Microbiol.*, 1965, **13** (1), 120
84. Jay, J. M., & Kontou, K. S., *ibid.*, 1964, **12** (4), 378
85. Jay, J. M., *Hlth. Lab. Sci.*, 1966, **3** (2), 101
86. Spinelli, J., Eklund, M., & Miyauchi, D., *J. Fd Sci.*, 1964, **29,** 710
87. Patashnik, M., *Comml Fish Rev.*, 1966, **28** (1), 1
88. Howard, A., Lee, C. A., & Webster, H. L., 'Studies on beef quality IX', Division of Food Preservation Technical Paper No. 21 (Melbourne, Australia: C.S.I.R.O.)
89. Stahl, W. H., 'Chemistry of natural food flavors' (Chicago, U.S.A.: Quartermaster Food and Container Institute for the Armed Forces)
90. Merritt, C., Bresnick, S. R., Bazinet, M. L., Walsh, J. T., & Angelini, P., *J. agric. Fd Chem.*, 1959, **7,** 784
91. Hornstein, I., & Crowe, P. F., *J. Gas Chromat.*, 1964, **2,** 128

APPLICATION OF CHEMICAL METHODS FOR THE ASSESSMENT OF BEEF QUALITY

I.—General considerations, sampling and the determination of basic components

By D. PEARSON

This paper introduces a series of contributions covering the application of chemical methods to samples of stored comminuted beef. The criteria to be investigated are outlined, together with the methods employed for sampling, determination of basic composition and assessment of odour.

Introduction

The microbiological, physical and biochemical changes which take place in meat during storage have been studied by several workers.[1] Most of the published work has been related to studies of changes in individual meats rather than the consideration of the results on a more general basis. Further, there has been little emphasis on the relationship between the objective results obtained and acceptability. As meat spoilage is influenced considerably by the initial and invading micro-organisms, it would be most desirable if recognised numerical limits based on bacterial and mycological counts could be laid down and applied in control systems. The suggested limits for the bacterial count in minced beef have varied widely, from 250×10^3 to 100×10^6 per g^2. Apart from the difficulty of deciding on a critical limit of acceptability which can be used with confidence, other problems arise when the more reliable microbiological techniques are applied in routine control work, e.g. lack of reproducibility, contamination during sampling, and the lengthy time of incubation. In general, chemical methods do not suffer from these disadvantages to such an extent.

Although some workers have followed changes which occur during the storage of beef, Jensen[1] and Turner[3] inferred that, with the possible exception of the pH value, no chemical method could be successfully employed for assessing meat spoilage. More recently, however, Jay[4,5] has recommended possible critical limits for beef acceptability based on the determination of the extract-release volume. In the fishery research field, it had been found that the amounts of volatile base and trimethylamine increased as white fish spoiled, and the chemical indices were successfully correlated with subjective sensory scores measured consistently by a trained laboratory panel, which is more used to examining much fresher material than the majority of private and industrial consumers are able to obtain. In practice, however, not only did the correlation become less signficant when results of white fish from different fishing grounds were examined, but the critical limits originally recommended had to be modified for application in industrial control.[6–8] The successful development of a chemical method with beef would represent a significant advance, as the comparatively high fat content presents a more complex system than with white fish. Further it is important to assess the feasibility of any recommended limits by comparing them with those found in beef sold in retail establishments.

Fresh meat after dressing contains few micro-organisms, but handling, transport, cutting and mincing may all contribute to their introduction. Although the original contaminating flora is diverse, it is usual for one organism to become eventually predominant, depending on various factors such as storage temperature, pH and the availability of nutrients, oxygen and water. The psychrophiles most commonly found on meat are the obligate aerobes, the pseudomonads, which have a high water requirement. Apart from microbial metabolism, the changes in the free amino acid levels can be brought about by muscle autolysis. Under aerobic conditions protein de-amination is likely to be the predominant action, but anaerobic conditions may encourage amine production by decarboxylation. In the later stages of spoilage, foul-smelling compounds such as indole and skatole result from the actions of bacterial enzymes. The overall effect of these reactions is the production of various volatile compounds with distinctive odours.

Spoilage of fat is mainly caused by oxidation of unsaturated bonds, hydrolysis due to attack by micro-organisms, free acidity production due to tissue enzymes and the acquirement of taints. The overall effect of these reactions is the production of compounds which cause deterioration in the odour and flavour.

The methods employed in the present investigation can be classified as follows:

Methods related to the breakdown of protein.

Methods related to fat spoilage.

Miscellaneous or general methods not related so specifically to the breakdown of protein or fat.

In general the analytical methods employed in this investigation for the possible assessment of spoilage of stored minced beef were considered in relation to agreement between replicates, the attainment of good recoveries, baseline consistency between different fresh samples, sensitivity ratio, i.e. the ratio of the values at the fresh and spoilage stages (Fig. 1), rapidity in performance, and correlation with organoleptic findings.

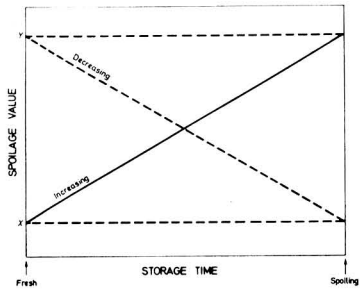

FIG. 1. *Diagram showing basis of calculation of 'sensitivity ratio' for objective methods used for assessing spoilage*

$$\text{Sensitivity ratio} = \frac{y}{x}$$

Experimental

Preparation of samples

In order to obtain consistent results, it is important that the samples used are homogeneous, so that small portions removed for analysis are representative of the original material. The special problems which arise in preparing such samples with meat arise due to its heterogeneity, the comparatively rapid loss of moisture and the spoilage changes that occur at room temperature.

Raw meat consists essentially of lean muscle and fatty tissue held together by a skeleton of tough connective tissue. The heterogeneity and the tendency of the connective tissue to clog the mincer combine together to make it probably more difficult to obtain an even sample with meat than with any other food.

Early work indicated that the most even mixing could be achieved by employing a 3 in. Multipurpose Colloid Mill (Premier Colloid Mills). Also Benne, Van Hall & Pearson[9] have described the use of a motor-driven grinder fitted with a fine-mesh cutting plate and divider, and good agreement between replicate results of fat, water and protein was reported after the sample had been ground and mixed six successive times. As such apparatus is not commonly to be found in laboratories, other methods using more conventional equipment were examined.

Laboratory blenders of the MSE Atomix and Waring type were found to be unsatisfactory as they tended to make the meat knit together rather than to cut through it. The more laborious method of successive mincing and mixing recommended by the former Society of Public Analysts[10] was then examined in more detail. In general, hand-operated mincers were used for the smaller samples and an electrically operated Kenwood model for large ones:

Method 1. Preparation of sample from minced beef

The minced beef was comminuted in either a hand- or electrically-operated mincer and then mixed with a pestle in a large mortar (10 in. diam.). Then the mincing and mixing operations were repeated at least once more until a desirable degree of comminution was achieved.

When the starting material was a whole piece of beef the following modification was employed:

Method 2. Preparation of sample from a piece of beef

The piece of meat was cut into cubes (approx. 2 in. square) by means of a sharp butcher's knife before being passed through an electrically operated industrial type mincer. The minced meat was then mixed in a mortar and the operations were repeated as in Method 1.

The *degree of homogeneity* achieved by these techniques was assessed by examining portions drawn from different areas of the sample. The protein content (Method 5 below) was determined in duplicate on 4–5 such portions. The coefficients of variation of 11 samples examined ranged from 0·5–4·2%.

The water, fat and protein contents were determined by the following conventional methods:

Method 3. Determination of water

Five g of comminuted sample were weighed into a dried metal dish (7 cm dia.) containing a flat-ended rod. After a little alcohol had been added the sample was spread by the rod and placed on a boiling water bath for about 10 min. The dish and rod + sample were then dried to constant weight at 100° in an oven in which the air was circulated by a fan.

Method 4. Determination of fat

The dried residue obtained from Method 3 was transferred to a Soxhlet apparatus and the fat was extracted with light petroleum (b.p. 40/60°C). During the extraction the material was removed, reground and returned to the extractor.

Method 5. Determination of crude protein

About 1·0–1·5 g of comminuted sample was employed for the macro-Kjeldahl procedure.[11] A factor of 6·25 was used for converting the total nitrogen to crude protein.

The basic composition of the samples of beef employed in the investigation are given in Table I.

TABLE I

Basic compositions of beef (61 samples) obtained from slaughterhouses and retail establishments

Determination	Percentages		
	Min.	Max.	Mean
Water	45·0	68·5	55·0
Fat	5·7	38·2	24·2
Protein (N × 6·25)	15·0	24·8	19·6
Nitrogen in fat-free material	3·63	4·66	4·15

Method 6. Assessment of odour

The organoleptic acceptability of fresh and stored samples was judged from the odour. An 8–10 g portion was removed from the stored sample and transferred to a clock glass or Petri dish. The sample was allowed to warm up to the laboratory temperature (for about 20 min) before presentation to a panel for assessment of the odour using the following scale:

Raw odour score	*Condition/acceptability*
10	Very fresh
9	Fresh
8	Fairly fresh
7	Acceptable
6	Just acceptable

5	Just spoiled
4	Unacceptable
3	Well spoiled
2	Nearly putrid
1	Putrid

Handling of samples

Beef in various forms was used as the starting material for storage tests:

Large pieces obtained from carcasses of animals, cut off immediately after dressing at the slaughterhouse.

1½–4 lb pieces of beef, with varying proportions of fat, obtained from retail butchers.

Minced beef from retail butchers and supermarkets.

Only material adjudged to be fresh and of good general quality was employed for samples used in storage tests. For greater consistency all stored samples were reduced to about the same degree of comminution, approximately similar to the finest obtainable from butchers and containing small visible pieces of fat. The pieces of meat used were first cut by a sharp knife before successive comminution (see also Method 2).

The fresh sample was distributed into several small jars, which were all stored on the same shelf of an incubator controlled at +5°. Then on each selected day for examination a jar was removed and the sample was re-comminuted by Method 1. In order to reduce the effect of differing aerobic conditions the jars in each storage experiment were filled to approximately the same level.

National College of Food Technology,
(University of Reading),
Weybridge, Surrey

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References

1. Jensen, L. B., 'Microbiology of Meats', 1954, 3rd edition (Garrard Press: Illinois)
2. Elliott, R. P., & Michener, H. D., *Appl. Microbiol.*, 1961, **9**, 452
3. Turner, A., *Fd Mf.*, 1960, **35**, 386
4. Jay, J. M., *Fd Technol., Champaign*, 1964, **18** (10), 133
5. Jay, J. M., & Kontou, K. S., *Appl. Microbiol.*, 1964, **12** (4), 378
6. Ehrenberg, A. S. C., & Shewan, J. M., *J. Sci. Fd Agric.*, 1955, **6**, 207
7. Shewan, J. M., & Ehrenberg, A. S. C., *J. Sci. Fd Agric.*, 1957, **8**, 227
8. Pearson, D., *Lab. Pract.*, 1962, **11**, 203
9. Benne, E. J., Van Hall, N. H., & Pearson, A. M., *J. Ass. off. agric. Chem.*, 1956, **39**, 937
10. Society of Public Analysts, *Analyst, Lond.*, 1952, **77**, 543
11. Pearson, D., *Lab. Pract.*, 1958, **7**, 652

APPLICATION OF CHEMICAL METHODS FOR THE ASSESSMENT OF BEEF QUALITY

II.* Methods related to protein breakdown

By D. PEARSON

Various methods for assessing the extent that the protein has spoiled in stored beef are considered in relation to the various criteria discussed in Part I. From the point of view of routine quality control, the macro-distillation of the volatile nitrogen produced appears to be the most reliable method. From statistical correlations with odour scores, most beef can be considered to be acceptable if the total volatile nitrogen (TVN) and TVN/FF (fat-free) figures do not exceed 16·5 and 19·7 mg N/100 g respectively.

Introduction

One of the more obvious effects of meat spoilage is the breakdown of the protein. The actual reactions which take place, however, vary according to the bacterium, the temperature and whether the conditions are aerobic or anaerobic. It is generally accepted that the psychrophilic bacteria growing on and causing spoilage in beef are mostly *Pseudomonas*, which frequently cause the production of ammonia by de-amination of amino acids under aerobic conditions.[1] Although beef after comminution is liable to contain a vague mixture of organisms, there would appear to be a greater possibility of bacterial de-amination than for other types of reaction to occur. Therefore, it seemed appropriate and advisable to apply to stored beef methods which measured production of alkalinity. Some of the earlier workers measured the extent of ammonia production in meat by macro-distillation from magnesium oxide,[2] aeration[3] and by addition of Nessler's reagent.[4] Many workers have studied the determination of the volatile nitrogen in fish, which frequently contains appreciable quantities of amine in addition to ammonia. Such volatile compounds in fish have been determined by macro-[5] and semi-macro[6]-distillation

* Part I: Previous paper

and micro-diffusion.[7] By employing g.l.c., Burks *et al.*[8] found that the volatiles from raw beef consisted almost entirely of ammonia.

Some workers have estimated the free amino nitrogen in meat, but Broumand, Ball & Stier[9] stated that the production is not always pronounced or consistent. Also only slight increases in the 'ninhydrin-positive substances' (alpha-amino acids) have been reported during spoilage.[10] Turner[11] stressed that determinations of indole and hydrogen sulphide only show significant increases over the fresh material when spoilage is advanced.

The degree of autolytic and bacterial proteolysis has been assessed in fish by means of the 'tyrosine value' of Bradley & Bailey.[12] In the presence of tyrosine, Folin & Ciocalteu's reagent produces a blue colour, the intensity of which is a measure of protein cleavage. In addition to tyrosine, the reaction measures tryptophan, cysteine, phenolic groups, sulphydryl compounds, hydrogen sulphide, etc.

Experimental

Particularly in view of work carried out previously on fish, the following methods were applied to beef stored at 5° and considered in relation to the criteria outlined in Part I.

Volatile nitrogen by macro-distillation under controlled conditions using magnesium oxide (Method 7).

Volatile nitrogen by semi-micro distillation from an alcoholic extract using sodium and barium hydroxides (Method 8).

Volatile nitrogen by micro-diffusion in a Conway unit using potassium carbonate (Method 9).

Tyrosine value, applying Folin & Ciocalteu's reagent to a de-proteinated TCA extract (Method 10).

Method 7. Volatile nitrogen (macro-distillation)

The following procedure was modified from that described by Lucke & Geidel:[5]

A macro-Kjeldahl distillation apparatus was set up, and to the 1 litre distilling flask was added 10 g prepared minced sample, 2 g magnesium oxide and 300 ml tap water. To the 500 ml receiving-flask was added 25 ml 2% boric acid solution and screened methyl red indicator. After the apparatus had been connected, the flask was heated so that the liquid boiled in exactly 10 min and, with the same rate of heating, the distillation was allowed to proceed for 25 min. When the condenser had been washed down with distilled water, the distillate was titrated with 0·1 N sulphuric acid. After subtraction of the blank (0·05 ml) the volatile nitrogen was calculated as mg N/100 g minced sample.

Recoveries were checked by adding known amounts of ammonium chloride.

Method 8. Volatile nitrogen (semi-micro distillation)

The following procedure was modified from that described for fish by Ronold & Jakobsen:[6]

10 ± 0·05 g of prepared minced sample was weighed into the 100 ml vortex container of an MSE homogeniser. After adding 35 ml alcohol (95%) the mixture was macerated mechanically at full speed for 2 min and transferred to a 50 ml volumetric flask. The residue was then washed into the flask with more alcohol and, after making up to the mark, the extract was filtered. 10 ml of filtrate were then pipetted into a semi-micro Kjeldahl distillation apparatus[13] together with 5 ml saturated barium hydroxide solution and 10 ml aqueous 2 N sodium hydroxide solution. 10 ml 0·02 N hydrochloric acid (previously standardised in 50% ethyl alcohol–water against 0·02 N sodium hydroxide using rosolic acid) were pipetted into the receiving flask and then the alkaline mixture was steam distilled for 10 min. The distillate was then titrated with 0·02 N sodium hydroxide using rosolic acid as indicator and the volatile nitrogen calculated as mgN/100 g sample.

Recoveries were checked by adding known amounts of ammonium chloride.

Method 9. Volatile nitrogen (micro diffusion technique)

The procedure employed was essentially that described for fish by Beatty & Gibbons[7] and Ehrenberg & Shewan.[14] It also incorporated, however, the modifications of Pearson[15] and some of the precautions mentioned by Spinelli:[16]

Boric acid reagent 100 ml alcohol (95%) was stirred with 5 g boric acid and 350 ml water added. After the acid had dissolved, 5 ml indicator (0·066% methyl red and 0·033% bromocresol green in alcohol) was added. Alkali was added until a faint reddish colour was produced, and the mixture was made up to volume with alcohol to 500 ml.

Procedure

50–100 g of prepared minced sample were thoroughly mixed in a porcelain basin with 2·5–5 g powdered trichloracetic acid using a kitchen fork. The mixture was allowed to stand for 30 min before filtering on a Buchner funnel. The filtrate was then re-filtered through a Whatman No. 5 paper using an ordinary filter funnel.

The filtrate was stored in a 1 oz screw-capped bottle at 0° until required. For the diffusion, which was normally carried out in triplicate together with a blank, 2 ml of the boric acid solution were added to the centre compartment of a Conway micro-diffusion unit. Then 1–2 ml of meat filtrate (or, with spoiled samples, a 2 : 10 dilution of it) was accurately pipetted into the outer compartment. After sliding the lid (smeared with Dow-Corning grease) so that only a small portion of the outer compartment was exposed, 1 ml of saturated potassium carbonate solution was added by pipette through the gap. The lid was quickly slid on to form an airtight seal, the dish was then rotated mechanically on a Gallenkamp Oscillating Table (ME-500) and the volatile nitrogen was allowed to diffuse over for 3 hours at 37°. Then the centre compartment was titrated with 0·02 N sulphuric acid and the total volatile nitrogen (TVN) was calculated as mg N/100 ml juice.

Recoveries were checked by adding known amounts of ammonium chloride.

Method 10. Tyrosine value

The procedure was essentially that described by Wood, Sigurdsson and Dyer:[17]

Two grams of prepared minced sample were weighed out into the 100 ml vortex beaker of an MSE Homogeniser and 40 ml of 5% trichloracetic acid solution were added. After homogenisation for 2 min the macerated mixture was filtered. 5 ml of filtrate (or a dilution of it) were then shaken with 10 ml of 0·5 N sodium hydroxide and 3 ml of diluted Folin & Ciocalteu's reagent (1 vol concentrated reagent + 2 vols water). After standing for 5 minutes, the optical density was measured in the 1 cm cell of a Unicam SP600 at 660 nm. By reference to the standard graph the tyrosine value was calculated as mg tyrosine/100 g sample.

Standard graph 0·100 g tyrosine were dissolved in 5% trichloracetic acid in a 500 ml volumetric flask and the solution was then made up to the mark with water. The following volumes of tyrosine solution were then added to a series of 100 ml volumetric flasks: 0, 1, 3, 5, 7, 10, 12, 15, 20 ml. Each was made up to the mark with water and mixed. 5 ml of each solution were shaken with sodium hydroxide solution and diluted Folin & Ciocalteu's reagent and then treated as described as for the determination above. The standard graph (Fig. 1) was prepared by plotting optical density against mg tyrosine/100 g sample (assuming that 2 g were used).

Recoveries were checked by adding known amounts of tyrosine dissolved in trichloracetic acid solution.

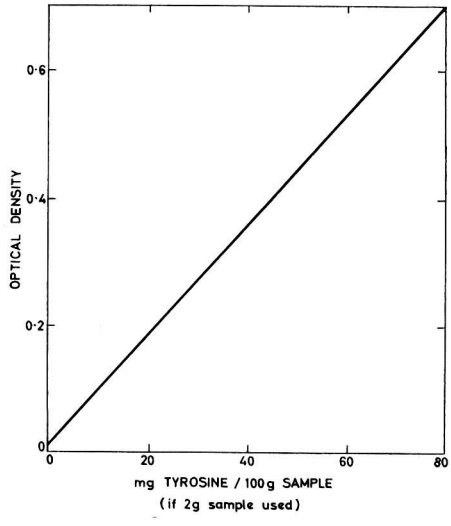

FIG. 1. *Standard graph employed for estimation of tyrosine value*

Results and Discussion

In general all the four methods employed showed increased values as spoilage advanced. Typical trends in the volatile nitrogen figure and the tyrosine value are shown in Fig. 2.

An examination of the date in Table I indicates the relative reliability for assessing spoilage of the four methods considered for following the course of protein breakdown. The figures used for the compilation of Table I were obtained from 11 samples which were stored at 5°. The values were determined firstly on the material as received, but only those meats which were adjudged to be fresh by the odour panel were used. Then the results were repeated when the stored comminuted samples were assessed as 'just spoiling'. So apart from showing such important criteria as the coefficient of variation between replicates, percentage recoveries and time of performance, Table I also shows the sensitivity ratio, which indicates to what extent the figures rise as the meats passed from fresh to spoilage (see Part I). The approximate

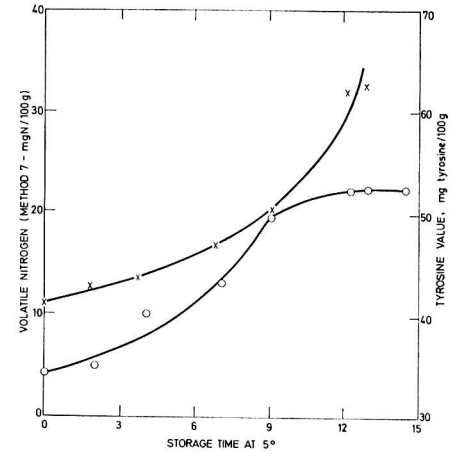

FIG. 2. *Typical changes shown by the TVN figure and tyrosine value as spoilage advances in beef stored at 5°*

TVN ×——×; TYRO ○——○

sensitivity ratios for the three TVN methods are about 2, which represents a satisfactory rise for assessment purposes. From the point of view of time of performance, Methods 7 and 8 are superior to the Conway technique, which gives, in addition, lower recoveries. In view of the good agreement between replicates and general simplicity in performance, the Lucke & Geidel macro-distillation Method (7) was preferred to the other techniques used. With the tyrosine value, apart from the lower sensitivity ratio, the wide variation of values obtained with fresh samples (34–66; coeff. of variation 31·6%) suggests that the determination is less likely to be suitable for the application of critical limits.

As a result of this earlier work, the TVN figure was determined by the modified Lucke & Geidel technique (Method 7) on a large number of stored samples, together with the odour assessment (Method 6—Part I). The correlation between the two results is shown graphically in Fig. 3. As the proportion of total nitrogen available for breakdown varied with the fat content, the TVN/FF (TVN in the fat-free sample) vs. odour score was also calculated (Fig. 4). By considering these relationships, the appropriate odour score can be calculated from the following formulae:

$$\text{Odour score} = -4{\cdot}02 \log_e(\text{TVN}) + 18{\cdot}24 \quad (r = -0{\cdot}83)$$

$$\text{Odour score} = -4 \log_e(\text{TVN/FF}) + 18{\cdot}91 \quad (r = -0{\cdot}85)$$

From the correlations it would appear that beef with the 'acceptable' odour 7 would correspond to TVN/FF and TVN figures of 19·7 and 16·5, respectively. Although these maxima would not necessarily apply for all the differing requirements in industrial practice (e.g. higher figures might be allowed for petfoods), they should represent a useful guide for initial work. Further, in view of the reasonably low coefficient of variation between fresh meats and the good sensitivity ratio, etc., of the macro-distillation technique, the method has been shown to have the attributes required for control purposes irrespective of where the limits might be

TABLE I

Data comparing the reliability of 5 different methods related to protein breakdown for assessing the degree of freshness of beef
(as applied to 11 samples stored at 5°)

Method	Coefficient of Variation between replicates (mean)	Recovery % (mean)	Values obtained with samples as received (fresh)				Values obtained at 'just spoiling' stage (mean)	Sensitivity Ratio 'just spoiling' value / 'fresh' value (mean)	Time of performance, h[a]	
			min.	max.	mean	C.V.(%)			1 determination	4 determinations
7. TVN (macro distillation)[b]	5·0	96·5	9·8	16·3	12·8	12·8	24·6	1·92	¾+	1½
8. TVN (semi-micro)[b] distillation)[b]	8·2	98·8	12·5	18·4	13·8	14·4	28·3	2·05	¾−	2
9. TVN (Conway micro diffusion)[c]	7·7	92·6	8·4	20·2	15·1	21·8	32·0	2·12	4½	5½
10. Tyrosine value[d]	4·4	99·0	34	66	47·7	31·6	69·2	1·54	½	1½

Notes: a Excluding time required for sample preparation
b Results expressed as mg N/100 g
c Results expressed as mg N/100 ml meat juice
d Results expressed as mg tyrosine/100 g.

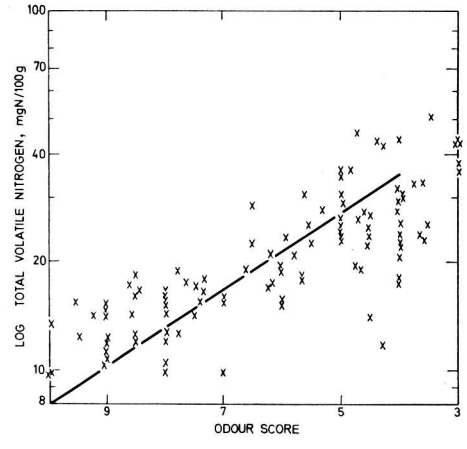

FIG. 3. *Graph showing correlation between the logarithm of TVN and odour score for samples of beef assessed when fresh and after storage at 5°*

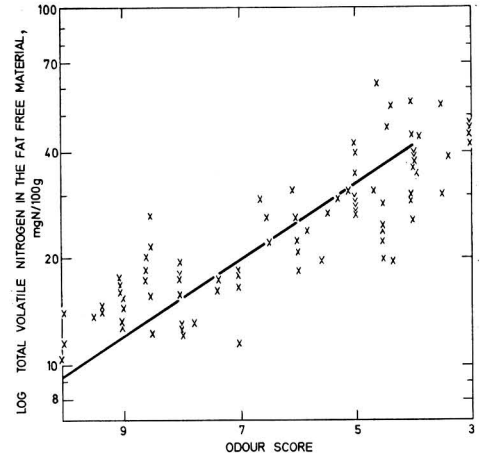

FIG. 4. *Graph showing correlation between the logarithm of TVN/FF and odour score for samples of beef assessed when fresh and after storage at 5°*

considered to fall by individuals, It must be borne in mind also that a few of the more fatty samples examined (containing > 25% fat) showed spoilage in the fat rather than in the lean tissue. The additional limits which have to be applied to such meats will be discussed in Part IV.

National College of Food Technology,
(University of Reading),
Weybridge, Surrey

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References

1. Gardner, G. A., *J. appl. Bact.*, 1965, **28,** 252
2. Richardson, W. D., & Scherubel, E., *J. Am. chem. Soc.*, 1908, **30,** 1515
3. Falk, K. G., & McGuire, G., *J. biol. Chem.*, 1919, **37,** 547
4. Falk, K. G., *J. Ass. off. agric. Chem.*, 1924, **8,** 160
5. Lucke, F., & Geidel, W., *Z. Unters. Lebensmittel*, 1935, **70,** 441
6. Ronold, O. A., & Jakobsen, F., *J. Soc. chem. Ind., Lond.*, 1947, **66,** 160
7. Beatty, S. A., & Gibbons, N. E., *J. biol. Bd Can.*, 1937, **3,** 77
8. Burks, R. E., Baker, E. B., Clark, P., Esslinger, J., & Lacey, J. C., *J. agric. Fd Chem.*, 1959, **7,** 778
9. Broumand, H., Ball, C., & Stier, E. F., *Fd Technol., Champaign.*, 1958, **12,** 159
10. Saffle, R. L., May, K. N., Hamid, H. A., & Irby, J. D., *Fd Technol., Champaign*, 1961, **15,** 465
11. Turner, A., *Fd Mf.*, 1960, **35,** 386
12. Bradley, H. C., & Bailey, B. E., *Fd Res.*, 1940, **5,** 487
13. Yuen, S. H., & Pollard, A. G., *J. Sci. Fd Agric.*, 1953, **4,** 490
14. Ehrenberg, A. S. C., & Shewan, J. M., *J. Sci. Fd Agric.*, 1955, **6,** 207
15. Pearson, D., *Lab. Pract.*, 1962, **11,** 538
16. Spinelli, J., *Fishery Ind. Res.*, 1964, **2** (3), 17
17. Wood, A. J., Sigurdsson, G. J., & Dyer, W. J., *J. Fish. Res. Bd Can.*, 1942, **6,** 53

ACTION OF GLUTARALDEHYDE, NITROUS ACID OR CHLORINE ON WHEAT PROTEINS

By J. A. D. EWART

The amino acid analyser has been used to determine which amino acid side chains (except that of tryptophan) in wheat proteins are attacked by three reagents. Glutaraldehyde crosslinks gliadin by reacting with lysine and tyrosine residues so that it gives an electrophoretic pattern resembling that of glutenin. Wheat gliadin after treatment with nitrous acid suffers an overall reduction in electrophoretic mobility due to deamination of lysine side chains. Chlorine oxidises part of the cystine and methionine of wheat flour protein to cysteic acid and the sulphone respectively, destroys a fraction of the tyrosine and histidine, and causes some deamidation probably as a result of hydrolysis by hydrochloric acid. The results suggest that there may be buried lysine and tyrosine residues in, and some degrees of structural similarity among, the gliadin proteins. Additional support for the crosslinked nature of glutenin is provided.

Introduction

The automatic amino acid analyser, introduced by Spackman *et al.*,[1] proved to be a helpful tool for studying reactions of proteins. For example Gundlach *et al.*[2] used it to show that the main action of iodacetate on ribonuclease was to attack lysine at pH 8·5–10, histidine at pH 5·5–6, and methionine at pH 2·8. Stark *et al.*[3] obtained evidence from the analyser for the conversion of lysine residues to those of homocitrulline in ribonuclease by reaction with traces of cyanate present in urea.

This paper describes attempts to ascertain the residues in wheat proteins which are attacked by glutaraldehyde, nitrous acid and chlorine.

Glutaraldehyde was reported by Cater[4] to be the best of the crosslinking agents for leather used by him; he suspected that lysine was the reaction site. In order to further test the hypothesis of Woychik *et al.*[5,6,7] that glutenin is made up of gliadin components crosslinked to one another by S.S bonds, the effect of a known crosslinking reagent on gliadin was examined.

By the action of nitrous acid it was hoped to modify gliadin proteins by replacement of $-NH_2$ groups by $-OH$ and cause changes in their electrophoretic mobilities which might throw light on their structure.

Chlorine is used industrially at a level of ~0·1% (5 oz per 280 lb sack) to produce certain types of special cake flours which can be mixed with higher quantities of liquid and sugar as in the 'high ratio' cake recipes. It would be of interest to understand more of the reaction.

Experimental

The wheat proteins studied in this paper and the flour subjected to chlorine treatment were produced from single wheat varieties of U.S.A. origin. This choice was made to avoid the ambiguities that would be introduced by using wheat and flour from British sources where a mixed grist would inevitably lead to a less precise material for study. The flours were milled on a Buhler mill to 70–72% extraction. The Rescue flour had a protein content of 15·0% (corrected to 14% moisture content).

Gliadin for glutaraldehyde treatment

Gluten was washed by hand kneading from dough made from Wichita 1963 flour (120 g), mixed with the calculated quantities of liquids to give 200 g of ethanol-water (70 : 30 wt./wt. and including $\frac{2}{3}$ of the weight of the wet gluten as water) and subjected to high-speed stirring for 30 sec. After further magnetic stirring for 2 h the suspension was centrifuged for 30 min at 900 *g*, the bulk of the alcohol removed by rotary evaporation and the remainder by dialysis against 0·01 N acetic acid. The freeze-dried extract was magnetically stirred for 1 h with 100 ml of water, ultra-centrifuged at 45,000 *g*, and the supernatant liquor was freeze-dried.

Glutaraldehyde treatment

Wichita 1963 gliadin (250 mg) was dissolved with magnetic stirring for 15 min in 12·5 ml of 0·01 N acetic acid containing 12·5 mg of glutaraldehyde, added as a 25% solution. After standing for 22 h the solution had become cloudy and slightly yellow, whereas a similar solution without addition of glutaraldehyde remained clear. Both solutions were dialysed against 2l of 0·01 N acetic acid for 2 h before freeze-drying. During dialysis the cloudy solution became clear though still yellow.

Performate oxidation

The glutaraldehyde-treated gliadin and its control (10 mg) were dissolved in 2·5 ml of 98% formic acid, cooled in ice and reacted at 0° for 2·5 h with 5 ml of ice-cold freshly made performic acid. The mixtures were freeze-dried, mixed with a few ml of water and again freeze-dried.

Gliadin for nitrous acid treatment

Wichita 1962 flour (200 g) was stirred for 1·5 h with 800 ml of ethanol-water (70 : 30 wt./wt.). After centrifuging for 30 min at 900 *g* the supernatant liquor was dialysed *vs.* 0·01 N acetic acid to remove alcohol and freeze-dried. The material was extracted overnight with water, ultracentrifuged at 45,000 *g* for 1 h, and the supernatant liquor freeze-dried.

Nitrous acid treatment

A solution of Wichita 1962 gliadin (0·5 g) in 20 ml of

0·01 N acetic acid was divided into halves which were cooled in ice together with two 10 ml portions of 0·05 N hydrochloric acid. Sodium nitrite (0·17 g) was added to one portion of the hydrochloric acid and sodium chloride (0·17 g) to the other and each solution was mixed with 10 ml of gliadin solution. The mixtures containing precipitated protein were stirred in ice for 2 h, dialysed overnight against 0·01 N acetic acid and freeze-dried. These experiments were later repeated and the corresponding products combined by suspending in 0·01 N acetic acid (~15 ml) and freeze-drying.

Chlorination

Chlorine (1·1% of the flour weight, equivalent to 50 oz per 280 lb sack, i.e. about ten times the level used commercially) was passed into Rescue flour (450 g), which was mechanically agitated in a wooden box, over a period of at least 30 min. As the flour smelt strongly of the gas, in order to prevent further reaction of free chlorine at the temperature of 105° during the hydrolysis to amino acids, samples were spread in a thin layer in a vacuum desiccator containing sodium hydroxide pellets for 14 days. This flour, which no longer smelt of chlorine, was used for the analyses.

Analyses

Previously described methods were used to determine the amino acid compositions,[8] amide[8] and Kjeldahl nitrogen[9] contents, and starch-gel electrophoretic patterns.[10] Difficulty in obtaining satisfactory solutions of the nitrous acid-treated gliadins was overcome by dissolving in 8 M urea and dialysing against gel buffer (sodium lactate, pH 3·5). Gliadins (7–10 mg) were hydrolysed with ~1000× their weight of constant boiling hydrochloric acid; flours (23 mg) were hydrolysed with 15 ml of acid. At least two runs on the analyser were made for each sample and means are reported.

Results and Discussion

The results are set out in Table I.

Owing to the decomposition during acid hydrolysis, the action of the reagents on tryptophan residues was not studied.

No corrections for destruction or slow liberation of amino acids during hydrolysis were made because only differences between the control and treated were being sought. The recoveries of amino acid nitrogen are only approximate since the small samples taken for hydrolysis were not weighed on a micro-balance. At the time when the work on the gliadin was done the currently available sample of gliadin was used. The two samples differ slightly in protein distribution (Figs 1 and 2) which may partly explain their different amide content.

Significance of results

Experience of the repeatability of amino acid analyses led to the decision to reject any decrease after treatment of less than ~7%. In the case of proline where there is greater 'noise' on the 440 nm tracing and determination of the norleucine equivalent involves the smallest of the three peaks of norleucine, errors can be even greater. The + 10·6% difference for proline in the glutaraldehyde experiments cannot, of course, be due to the reaction, but to an unfavourable combination of random errors. The occurrence of a reaction was in each case obvious by the significant decrease.

Glutaraldehyde

Performic acid oxidation was used to destroy traces of glutaraldehyde remaining after dialysis,[11] so that further reaction of free aldehyde with the protein would not occur at the temperature of hydrolysis.

This reagent has attacked lysine and tyrosine residues (Table I). Calculation shows that the reaction mixture

TABLE I

	Glutaraldehyde (Residues per 1000 recovered residues)		% change after treatment	Nitrous acid (Residues per 1000 recovered residues)		% change after treatment	Chlorine (Residues per 1000 recovered residues)		% change after treatment
	Control	Treated		Control	Treated		Control	Treated	
$CySO_3H$	33·8	32·3	− 4·4	—	—	—	—	5·9	—
Met O_2	10·8	11·5	+ 6·5	—	—	—	—	7·2	—
Asp	27·4	27·5	+ 0·4	26·1	26·1	0·0	38·9	40·1	+ 3·1
Thr	24·4	24·2	− 0·8	25·2	25·5	+ 1·2	29·8	30·0	+ 0·7
Ser	61·8	58·9	− 4·7	63·0	63·0	0·0	58·2	60·9	+ 4·6
Glu	348·0	350·0	+ 0·6	349·4	359·9	+ 3·0	305·7	313·6	+ 2·6
Pro	153·1	169·3	+ 10·6	164·1	159·9	− 2·6	154·0	146·8	− 4·7
Gly	34·5	35·6	+ 3·2	35·2	36·3	+ 3·1	63·4	61·1	− 3·6
Ala	35·9	36·6	+ 1·9	35·4	34·8	− 1·7	42·4	42·8	+ 0·9
Val	42·6	43·7	+ 2·6	42·6	40·5	− 4·9	45·9	46·9	+ 2·2
CyS/2	—	—	—	20·7	21·8	+ 5·3	18·4	10·9	−40·8
Met	—	—	—	11·3	11·4	+ 0·9	6·0	1·9	−68·3
Ile	37·1	36·6	− 1·3	35·3	35·8	+ 1·4	36·2	37·0	+ 2·2
Leu	75·6	75·2	− 0·5	75·3	74·1	− 1·6	71·0	72·5	+ 2·1
Tyr	18·7	9·9	−47·1	22·2	22·1	− 0·5	23·7	16·2	−31·6
Phe	44·9	43·6	− 2·9	44·5	43·6	− 2·0	41·9	41·8	− 0·2
Lys	9·4	4·3	−54·3	8·5	4·9	−42·4	17·9	18·8	+ 5·0
His	18·4	18·3	− 0·5	18·9	17·9	− 5·3	18·5	15·4	−16·8
Arg	23·7	22·7	− 4·2	22·3	22·5	+ 0·9	28·1	30·1	+ 7·1
Total	1000·1	1000·2	—	1000·0	1000·1	—	1000·0	999·9	—
Amide	362·6	372·2	+ 2·6	311·0	330·6	+ 6·3	267·1	202·7	−24·1
Mean % recovery of amino acid N	88	100		90	87		92	82	

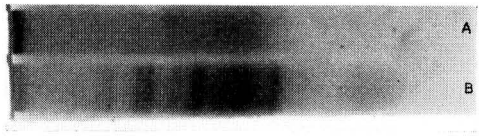

FIG. 1. *Starch-gel electrophoretic patterns showing action of glutaraldehyde on Wichita gliadin*

A – treated; B – control
Buffer, Na lactate pH 3·5
(The treated sample was not from the preparation which was analysed).

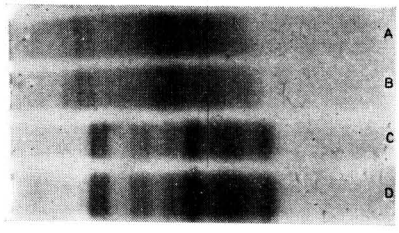

FIG. 2. *Starch-gel electrophoretic patterns showing effect of nitrous acid treatment on Wichita gliadin*

A,B – treated; C,D – control
Buffer, Na lactate pH 3·5

contained 205 mg of protein in which were present 12·55 and 38·25 μmoles of lysine and tyrosine residues respectively, giving a total of 50·8 μmoles.[8] Of these 24·8 μmoles reacted with glutaraldehyde of which there were 125 μmoles in the system. Since glutaraldehyde is difunctional there were 250 μmoles of $-$CHO groups of which 10% reacted. The probability of a given aldehyde group having reacted was therefore 0·1, and the chance that both ends of a glutaraldehyde molecule chosen at random have reacted is 0·01, which leads to an estimate of 1·25 μmoles of fully reacted glutaraldehyde. An average value of ~33,000 for the molecular weight of gliadin molecules (cf. Jones *et al.*[12]) would imply 6 μmoles of protein in the system, which with the above figure gives one cross link to every 5 molecules. This figure can only be regarded as an approximate measure of the number of intermolecular crosslinks; apart from the possibility of intramolecular crosslinking, it is not known whether the reactivity of the 2nd $-$CHO group is enhanced or decreased, once the 1st group has reacted, by steric and inductive effects.

A sample of the glutaraldehyde-treated gliadin gave only faint, discrete electrophoretic bands which appeared to be reduced in mobility compared with those of gliadin, a heavy mark on the starting line and slow tailing reminiscent of the criteria for glutenin[13,14] (Fig. 1). The last two would be expected if some large structures had begun to form and may indicate that the above figure for extent of crosslinking is an underestimate: the blocking of accessible lysine residues without crosslinking would have merely reduced the charge and given a pattern of more slowly moving bands, which has occurred to only a minor degree. When 1–2 mg of the gliadin were moistened with tap water and worked with the point of a needle, the crosslinked sample was appreciably more elastic than the control which possessed mostly viscous properties. This is further support for the cross-linked nature of glutenin brought to light by workers at Peoria.[12,13,15]

Condensation of glutaraldehyde with N-terminal α-amino groups would probably have occurred but would not have been detected in a mixture of proteins due to the small change in the quantities of the amino acids involved. This process would have increased the level of crosslinking above the calculated value.

Nitrous acid

Lysine appears to be the only acid which is significantly affected, about 40% being lost. It had been hoped that nitrous acid would remove amide groups, but these appear to be intact. A general reduction in electrophoretic mobility is seen (Fig. 2) with preferential loss of the leading gliadin band. It is known that increase of negative charge by one unit causes a change of mobility on a typical starch-gel electrophoretic run by one 'band spacing'.[16] This suggests that there is a regular loss of charge throughout the sample of probably two units per molecule. Table I shows that rather less than half the lysine residues have disappeared which could explain the mobility reduction; by calculations from earlier results,[8] Wichita gliadin possesses an average of 2 lysine residues in a molecular weight of 33,000 and Jones *et al.*[17] have given the molecular weight of purified γ-gliadin of Ponca variety as 31,000.

A loss of charge by N-terminal α-deamination of the polypeptide chains would also give a regular mobility change throughout the pattern and could escape detection unless the N-terminal amino acid were present in low concentration. A further possibility is that lysine itself is N-terminal and lost α- or ε-amino groups or both. This has not been reported, however, by Winzor & Zentner,[18] nor by Woychik *et al.*[5]

The most probable explanation is that there has been a loss of N-terminal α-amino groups together with a loss of one lysine throughout most if not all the gliadin molecules.

It is interesting that in the cases of glutaraldehyde and nitrous acid, which both attack lysine, although there was a large excess of reagent in each case (+300 μmoles of nitrous acid were available for the same quantity of gliadin as was used in the glutaraldehyde reaction), approximately half the lysine residues had reacted. (Rather less lysine reacted with nitrous acid in spite of the excess reagent, but this may be due to the heterogeneity of the reaction causing a small proportion of molecules to react incompletely. Support for this appears in the blurred nature of the bands in Fig. 2.) This suggests that all the lysine residues except one are buried deeply inside each molecule and are inaccessible even to small molecules. It would further suggest that there may be a basic similarity as regards this feature of one exposed lysine residue in the structure of the gliadins for them to react in a like manner, with the possible exception of the fastest gliadin component.

It is reasonable to infer by similar arguments applied to the glutaraldehyde reaction that there are buried tyrosine residues in at least some of the gliadin molecules.

Chlorine

The chlorinated flour was treated at about 10 $\times$ the level used in industry. About 40% of the cystine recovered in the

control was oxidised chiefly to cysteic acid in the treated flour: the word 'recovered' is used because losses of cystine during flour hydrolysis are large.

Approximately one third of the tyrosine and one sixth of the histidine was attacked. Tyrosine is well known to be substituted by halogens, and chlorine successively replaces the hydrogen atoms attached to the three carbon atoms of imidazole.[19] Ewart[20] observed that the loss of histidine could have been significant in a hydrolysate of performate-oxidised wheat flour when compared with an unoxidised control. There had been a severe loss of tyrosine, presumably due to chlorination, and it is interesting that these results confirm the significance of the histidine loss. Over 20% of the amide groups had been lost; probably hydrochloric acid formed during chlorination hydrolysed them to ammonia. In support of this the flour had a very high blank value during the amide determination and the moist flour had a strongly acid reaction.

Two thirds of the methionine was attacked by chlorine. On the analyser charts for the control flour there were peaks preceding aspartic acid in the section where methionine sulphoxide occurs, but they were not included because their areas were considered too large to be entirely due to methionine sulphoxide: they may well have been due to it but coincided with peaks due to artifacts which usually appear in this particular section and sometimes interfere with the peaks of the sulphoxide. It is worth noting that methionine appeared to be present as the sulphoxide in some cereal flours.[20] Probably therefore there was some sulphoxide present which was also oxidised to the sulphone, but as it was not included in the methionine total for the control flour there is an apparent total increase in methionine (unoxidised + sulphone) in the chlorinated flour (Table I).

A photograph of the electrophoretic pattern of a less heavily chlorinated flour has already been published.[21] The effect of the higher level of chlorine was to diminish the band intensities and to increase further the background tailing. Scission of S.S bonds would help to break down the protein structure, and in addition to the aforementioned reactions, labile peptide bonds would be ruptured as were amide bonds. In commercially treated flours these reactions would have occurred to only perhaps 10% of the extent found here, but would nevertheless be enough to cause marked weakening of the gluten. Chlorine will also react with starch and this sytem has been thoroughly investigated by Whistler and his co-workers.[22-24]

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Flour Milling and Baking Research Association,
Chorleywood,
Rickmansworth, Herts.

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References

1. Spackman, D. H., Stein, W. H., & Moore, S., *Analyt. Chem.*, 1958, **30,** 1190
2. Gundlach, H. G., Stein, W. H., & Moore, S., *J. biol. Chem.*, 1959, **234,** 1754
3. Stark, G. R., Stein, W. H., & Moore, S., *J. biol. Chem.*, 1960, **235,** 3177
4. Cater, C. W., *J. Soc. Leath. Trades Chem.*, 1965, **49,** 455
5. Woychik, J. H., Huebner, F. R., & Dimler, R. J., *Abstr. 47th A. Mtg Am. Ass. Cereal Chem.*, 1962, May, No. 61
6. Woychik, J. H., *Proc. Seed Protein Conf.*, (New Orleans, La.), Jan. 1963, 27
7. Woychik, J. H., Huebner, F. R., & Dimler, R. J., *Archs Biochem. Biophys.*, 1964, **105,** 151
8. Ewart, J. A. D., *J. Sci. Fd Agric.*, 1967, **18,** 111
9. Ewart, J. A. D., *J. Sci. Fd Agric.*, 1968, **19,** 241
10. Ewart, J. A. D., *J. Sci. Fd Agric.*, 1966, **17,** 526
11. Hickinbottom, W. J., 'Reactions of organic compounds', 1948, 2nd ed., 187, (London: Longmans Green & Co.)
12. Jones, R. W., Babcock, G. E., Taylor, N. W., & Senti, F. R., *Archs Biochem. Biophys.*, 1961, **94,** 483; 1964, **104,** 527
13. Woychik, J. H., Boundy, J. A., & Dimler, R. J., *Archs. Biochem. Biophys.*, 1961, **94,** 477
14. Elton, G. A. H., & Ewart, J. A. D., *J. Sci. Fd Agric.*, 1964, **17,** 34
15. Nielsen, H. C., Babcock, G. E., & Senti, F. R., *Archs Biochem. Biophys.*, 1962, **96,** 252
16. Feinstein, A., *Nature, Lond.*, 1966, **210,** 135
17. Jones, R. W., Babcock, G. E., & Dimler, R. J., *Cereal Chem.*, 1965, **42,** 210
18. Winzor, D. J., & Zentner, H., *J. Sci. Fd Agric.*, 1962, **13,** 428
19. Karrer, P., Organic Chemistry, 1947, 3rd English edition, 774, (Amsterdam: Elsevier Publishing Co. Inc.)
20. Ewart, J. A. D., *J. Sci. Fd Agric.*, 1967, **18,** 548
21. Elton, G. A. H., & Ewart, J. A. D., *J. Sci. Fd Agric.*, 1964, **15,** 119
22. Uchino, N., & Whistler, R. L., *Cereal Chem.*, 1962, **39,** 477
23. Ingle, T. R., & Whistler, R. L., *Cereal Chem.*, 1964, **41,** 474
24. Whistler, R. L., Mittag, T. W., & Ingle, T. R., *Cereal Chem.*, 1966, **43,** 362

INFLUENCE OF DIFFERENT YEASTS ON THE GROWTH OF LACTIC ACID BACTERIA IN WINE

By J. C. M. FORNACHON

The influence of various yeasts on the growth of lactic acid bacteria in wine was tested by inoculating *Lactobacillus hilgardii*, *L. brevis* and two strains of *Leuconostoc mesenteroides* into experimental wines made with twelve different yeasts of the genus *Saccharomyces*. Wines made from juice which had been infected with several spoilage yeasts and then fermented with a wine yeast were also tested in this way. It was found that the yeasts differed considerably in their effects on bacterial growth. In some of the experimental wines bacterial growth was delayed or failed altogether. Generally, the unfavourable influence of any yeast on bacterial growth was much reduced if the wines were left in contact with the yeast cells for some weeks after the fermentation.

The significance of these results in relation to the occurrence of malo-lactic fermentation in commercial wineries is discussed.

Introduction

Wine is a less favourable medium than grape juice for the growth of most micro-organisms, for during fermentation the yeasts produce important quantities of alcohol and also deplete the liquid of various nutrients and growth factors. On the other hand, however, certain growth factors and amino acids may be liberated by the yeast cells towards the end of fermentation[1] or more particularly during subsequent autolysis of the yeast.[2] Thus a wine left in contact with the fermentation lees may gradually become more favourable for the growth of bacteria than a wine which has been separated from the yeast promptly after fermentation.[3,4] These opposing effects of yeasts on the growth of bacteria in wines have been discussed recently by Radler.[5]

Practical interest in this subject stems from the importance of certain lactic acid bacteria in wine either as spoilage organisms or in bringing about the decarboxylation of malic acid in the malo-lactic fermentation. In recent years there has been much interest in the promotion of malo-lactic fermentation in wines by the use of selected bacteria, and in general the most successful procedures have involved addition of bacteria to the fermenting must rather than to the finished wine.[6–8] However, even though the bacteria are added during the primary fermentation, the malo-lactic fermentation is seldom completed until some time after the yeast fermentation and sometimes this delay amounts to several weeks or even many months. Thus the bacteria responsible for the decomposition of the malic acid are likely to be exposed to both the inhibiting and the stimulating influences of the yeast.

Riberéau-Gayon & Peynaud[9] grew yeasts belonging to 15 different species in grape juice and found that 11 of these inhibited subsequent growth of two lactic acid bacteria. Milisavljevic[10] reported that in experimental wines made with two strains of yeast, the natural occurrence of malo-lactic fermentation was earlier after one yeast in each of five successive years. On the other hand, Webb & Ingraham[7] found that the bacteria used by them grew equally well with any of the five strains of wine yeasts which they used, and it was concluded that 'the particular yeast strain used does not have a profound effect on the malo-lactic fermentation'.

The results of some inoculations with bacteria in Australian wineries in recent years have suggested that the strains of yeast used in the fermentation may differ significantly in their effects on bacterial growth. Accordingly this subject has been studied experimentally and the results are presented in this paper.

Experimental

Yeasts

The yeasts were chosen from the culture collection of The Australian Wine Research Institute and included 11 wine yeasts and one brewery yeast, all belonging to the genus *Saccharomyces*, and also 6 yeasts, belonging to other genera, which sometimes occur as spoilage yeasts in grape juice or wine.

The wine yeasts had been specially selected for winery use because of particular characteristics and are used extensively in commercial winemaking. Further details of some of these are reported by Rankine.[11]

Bacteria

The strains of bacteria used were two of *Leuconostoc mesenteroides*, one of *Lactobacillus hilgardii*, and one of *Lactobacillus brevis*. The bacteria had all been isolated from wine in this laboratory and had all been found capable of bringing about a malo-lactic fermentation in wine.

Grape juice

Experiments were carried out with fresh grape juice in three successive seasons. In 1965, juices from the varieties Riesling and Doradillo were used. Riesling was used again in 1966 and Grenache was used in 1967. The Doradillo contained 19% sugar, the Grenache 20% and the Riesling juices 21%.

All the juices were adjusted to pH 3·6 with hydrochloric acid or sodium hyroxide and were sterilised by filtration into sterile bottles which were closed with one-holed rubber stoppers plugged with cotton-wool. In different experiments

375 ml or 750 ml bottles were used and contained 200 ml or 500 ml of the sterilised grape juice.

Procedure

The yeasts were prepared as active cultures in sterilised grape juice and these were used at the rate of 3% by volume to inoculate the bottles of filter-sterilised juice which were then incubated at 15° ± 1°C. All yeast fermentations were carried out in duplicate.

When yeasts belonging to genera other than *Saccharomyces* were used, fermentation was completed by *Saccharomyces cerevisiae* No. 348 added 14 days after the initial inoculation.

The influence of the different yeasts on bacterial growth was determined by inoculating portions of the wines with bacteria. The wines had been previously decanted from their lees, readjusted to pH 3·6 when necessary, and sterilised by filtration into sterile 1 oz McCartney bottles. Duplicate 15 ml portions of wines from every yeast fermentation were inoculated with each of the 4 bacteria using as inoculum 0·2% of an active 6 day culture grown in a medium consisting of grape juice diluted with an equal volume of 0·5% aqueous solution of yeast extract (Difco) and adjusted to pH 3·8. These bacterial growth tests were incubated at 22° and examined visually at weekly intervals for growth.

In one experiment the bacteria were inoculated in the juice as soon as fermentation had commenced and were allowed to grow with the yeasts during the primary fermentation. In this experiment 8 bottles, each containing 200 ml of juice, were treated with each yeast and then 2 of these were inoculated with each of the 4 bacteria. The activity of the bacteria was then followed by weekly chromatographic examination of the liquid for malic and lactic acids.

Results of experiments have been expressed as the number of weeks before the end of the experiment that bacterial growth became evident or malo-lactic fermentation was completed. Thus a value of zero indicates that no bacterial growth occurred, while high values indicate rapid growth.

Results

Growth of bacteria in wines made with different yeasts

An experiment was carried out with treatments arranged in a factorial design to allow comparisons to be made between 12 yeasts, 4 bacteria and 2 grape juices, between wines removed from their lees as soon as fermentation was complete (racked early) and wines left on their less for 8 weeks (racked late) and also between dry wines and wines which had received an addition of 1% invert sugar before inoculation with bacteria.

The results of this experiment are summarised in Table I, II and III. For simplicity in presentation, the results obtained with and without the addition of sugar to the wines have been pooled in Tables I and II which show the influence of different yeasts and of delaying racking on the growth of bacteria in wines made from two juices. The significance of second-order interactions between the various factors studied is shown in Table III.

Each of the variables studied had a significant effect on the growth of the bacteria ($P = <0{\cdot}05$). In most instances delayed racking stimulated growth. All of the bacteria grew more rapidly in the wines made from the Doradillo juice than in those made from the Riesling. All of the wines contained less than 0·2% reducing sugar when removed from the lees, and it was found that the addition of sugar before inoculation usually stimulated bacterial growth. However, added sugar had less influence than the other factors studied, and none of the interactions between sugar and other factors was statistically significant.

The alcohol content of the wines made from the Riesling

TABLE I

Influence of different yeasts on growth of bacteria in wines
Bacteria added after the yeasts were removed. Incubated 8 weeks

Yeast		Growth of bacteria						
		Riesling wine			Doradillo wine			Mean
		Racked early	Racked late	Mean	Racked early	Racked late	Mean	
138	*Saccharomyces fructuum*	1·2	3·2	2·2	6·2	5·7	5·9	4·1
213	*S. cerevisiae*	0·8	0·1	0·4	0	4·7	2·3	1·4
317	*S. chevalieri*	0·5	0·9	0·7	5·4	5·4	5·4	3·1
348	*S. cerevisiae*	0·7	3·1	1·9	5·2	6·4	5·8	3·9
350	*S. cerevisiae*	1·5	5·4	3·5	6·8	6·3	6·5	5·0
709	*S. cerevisiae*	3·1	4·6	3·8	5·6	6·0	5·8	4·8
710	*S. cervisiae* var. *ellipsoideus*	1·6	3·2	2·4	6·6	6·3	3·4	4·4
723	*S. oviformis*	0·7	0·2	0·4	0·6	5·2	2·9	1·7
727	*S. cerevisiae*	0·4	2·4	1·4	4·2	4·9	4·5	3·0
729	*S. cerevisiae*	1·1	0·9	1·0	4·6	5·6	5·1	3·0
730	*S. cerevisiae*	3·4	4·9	4·1	6·4	6·6	6·5	5·3
731	*S. carlsbergensis*	0	0·6	0·3	0	4·8	2·4	1·4
	Mean	1·2	2·5	1·85	4·3	5·7	5·0	

L.S.D. ($P<0{\cdot}05$) between means:

in body of table	1·05
means of each yeast	1·50
means for each wine	0·62
means for each racking date	1·20
means for yeast x wine	0·75

TABLE II

Growth of different bacteria in four wines
Bacteria added after yeasts removed and incubated for 8 weeks

Bacterium		Bacterial growth				
		Riesling wine		Doradillo wine		Mean
		Racked early	Racked late	Racked early	Racked late	
L107	*Lactobacillus hilgardii*	0·8	2·8	4·2	6·7	3·6
L115	*Lactobacillus brevis*	0	1·7	4·8	6·8	3·3
L168	*Leuconostoc mesenteroides*	3·4	3·3	4·9	6·5	4·5
L178	*L. mesenteroides*	0·8	2·1	3·4	2·7	2·2

L.S.D. ($p < 0·05$) between means
body of table 0·44
means of bacteria 1·70

TABLE III

Growth of bacteria in wines made with different yeasts and under different conditions

Significance of interactions between various factors influencing growth of the bacteria

Second-order interactions					significance
Yeast	×	bacterium	×	grape juice	**
Yeast	×	bacterium	×	sugar	N.S.
Yeast	×	bacterium	×	time on lees	**
Yeast	×	grape juice	×	sugar	N.S.
Yeast	×	grape juice	×	time on lees	***
Yeast	×	sugar	×	time on lees	N.S.
Bacterium	×	grape juice	×	sugar	N.S.
Bacterium	×	grape juice	×	time on lees	***
Bacterium	×	sugar	×	time on lees	N.S.
Grape juice	×	sugar	×	time on lees	N.S.

** significant at $p < 0·01$
*** significant at $p < 0·001$
N.S. not significant at $p < 0·05$

juice ranged from 11·5 to 12·1% v/v while in the wines made from Doradillo it ranged 9·8 to 10·5% v/v. Although it is likely that this difference in alcohol content was largely responsible for the more rapid growth of the bacteria in the Doradillo wines than in the Rieslings, no relationship was found between the rate of bacterial growth and the small differences in alcohol content of the wines made from one variety of juice with different yeasts.

Malo-lactic fermentation in the presence of different yeasts

The yeasts were present in the wines from the beginning of this experiment, and consequently it was not possible to estimate bacterial growth by visual inspection of the wines. Accordingly the course of malo-lactic fermentation was followed by examining all cultures by paper chromatography at weekly intervals. Since the criterion used to indicate bacterial activity was the completion of malo-lactic fermentation rather than the appearance of bacterial growth as in previous experiments, it was found necessary to extend the total period of incubation to 20 weeks.

The results of this experiment are summarised in Table IV. Again it can be seen that the strain of yeast has had an important influence on bacterial activity. One of the bacteria used (L178) brought about complete malo-lactic fermentation in only those wines made with yeasts 709 and 730. Consequently the results obtained with this bacterium have been omitted from Table IV.

Influence of some spoilage yeasts on bacterial growth in wine

Duplicate portions of Grenache grape juice were inoculated with each of six spoilage yeasts and one wine yeast (*S. cerevisiae*). Two weeks later, the wine yeast was also added to all the cultures previously inoculated with the spoilage yeasts. This second inoculation was to ensure that fermentation went to completion in all cultures. When visible fermentation had ceased and loss of weight was constant in all cultures the wines were decanted from the lees, sterilised by filtration into 1 oz McCartney bottles, and inoculated with bacteria as described previously.

The results of this experiment are summarised in Table V. It can be seen that some of these spoilage yeasts depressed the growth of the bacteria considerably. The interaction between yeasts and bacteria was statistically significant ($p = < 0·05$); this indicated that some spoilage yeasts inhibit the growth of some of the lactic acid bacteria much more than others. Overall the two species of *Pichia* and *Saccharomycodes ludwigii* had the greatest inhibitory action on the bacteria. It may be noted that the two strains of *Candida pulcherrima* had very similar effects on the growth of the bacteria tested, although these two yeasts were isolated from grapes grown in Spain and South Australia, respectively.

Discussion

The growth of bacteria in wine and the length of time taken for the completion of malo-lactic fermentation are influenced significantly by the strain of yeast used to carry out the fermentation. In some experiments bacterial growth failed completely in association with certain yeasts.

Of the yeasts tested, two strains of *Saccharomyces cerevisiae* (709 & 730) were the most favourable towards bacterial growth, while *S. carlsbergensis* (731) and *S. oviformis* (723) were the most antagonistic in all experiments. The order of the other yeasts varied slightly from one experiment to another.

In a separate experiment, the bacteria were grown in grape juice and in a partly synthetic medium containing 30% grape juice, both in the presence and in the absence of yeast No. 138. In the grape juice the bacteria grew more rapidly in the absence than in the presence of yeast, but in the partly

synthetic medium the reverse was true.

Undoubtedly, the alcohol produced during fermentation is a major factor in the antagonism of yeasts towards bacteria, but it is apparent that other factors are also concerned. The differences noticed between the effects of different yeasts on bacterial growth in the experiments described were not related to the amounts of alcohol produced by these yeasts, and the results presented in Table V show that some of the non-fermenting yeasts depressed bacterial growth markedly.

The ability of some yeasts to produce sulphur dioxide during fermentation may also be responsible for some of the antagonistic effect which these yeasts have towards bacteria. It has been shown recently by Rankine and Pocock in this laboratory (unpublished results) that two of the yeasts mentioned in Tables I and IV (723 and 729) produced significantly more sulphur dioxide than several other strains which they studied.

Generally the unfavourable influence of any yeast on bacterial growth was much reduced if the wines were left for a period in contact with the yeast cells in the lees after the fermentation. This effect was more marked with some strains of yeast than others, and differences observed were probably related to differences in the rate of autolysis of the different yeasts. Kulka[12] and Weinfurtner *et al.*[13] found that strains of brewery yeasts differ very widely in their rates of autolysis.

At present there is much interest in the promotion of malo-lactic fermentation in commercial wineries by seeding

TABLE IV

Influence of different yeasts on malo-lactic fermentation by bacteria added to fermenting grape juice
Total period of incubation 20 weeks

Yeast		Malo-lactic fermentation by different bacteria*			
		L107	L115	L168	Mean
138	*Saccharomyces fructuum*	4·0	2·0	14·5	6·8
213	*S. cerevisiae*	6·0	7·5	14·0	9·2
317	*S. chevalieri*	5·5	7·5	14·0	9·0
348	*S. cerevisiae*	1·5	8·0	12·0	7·2
350	*S. cerevisiae*	0	8·0	12·0	6·7
709	*S. cerevisiae*	10·0	12·0	15·5	12·5
710	*S. cerevisiae* var. *ellipsoideus*	3·0	9·0	12·5	8·2
723	*S. oviformis*	0	0	6·5	2·2
727	*S. cerevisiae*	4·0	7·0	12·0	7·7
729	*S. cerevisiae*	0	7·0	11·0	6·0
730	*S. cerevisiae*	12·0	12·5	15·0	13·2
731	*S. carlsbergensis*	0	0	5·5	1·8
	Mean	3·8	6·7	12·0	

L.S.D. ($p < 0·05$) between means:

body of table	2·48
means for yeasts	4·66
means for bacteria	2·35

* Bacterium L178 was also included in this experiment, but brought about complete malo-lactic fermentation only in wines made with yeasts 709 and 730 so was omitted from the statistical analysis

TABLE V

Influence of different spoilage yeasts on the growth of bacteria in wine
Bacteria added after the yeasts were removed and incubated for 10 weeks

Yeast		Growth of different bacteria				
		L107	L115	L168	L178	Mean
63	*Saccharomycodes ludwigii*	1·8	0·8	2·0	0	1·2
168	*Pichia* sp.	0·5	0·3	1·5	0·8	0·8
287	*Candida pulcherrima*	5·3	5·5	6·0	4·5	5·3
442	*Schizosaccharomyces ledevorans*	5·8	7·8	7·3	5·0	6·4
443	*P. membranaefaciens*	0	2·3	8·0	2·5	3·2
448	*C. pulcherrima*	6·5	5·8	6·5	4·3	5·0
348	*Saccharomyces cerevisiae*	7·5	7·5	6·3	6·5	7·0
	Mean	3·9	4·3	5·4	3·4	

L.S.D. ($0 < 0·05$) between means:

body of table	2·51
means for yeasts	2·04
means for bacteria	1·53

cultures of selected bacteria into fermenting must. This has been achieved in Australia and in several other countries, but generally the results have been unreliable. The results reported above indicate that the strain of yeast used to carry out the primary fermentation and the chance contamination of the must by various spoilage yeasts may play an important part in determining the success or failure of bacterial inoculation.

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The Australian Wine Research Institute,
Adelaide,
South Australia

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References

1. Thorne, R. S. W., *J. Inst. Brew.*, 1945, **51**, 6
2. Joslyn, M. A., *Wallerst. Lab. Comm.*, 1955, **18**, 107
3. Fornachon, J. C. M., 'Bacterial spoilage of fortified wines', 1943, Australian Wine Board, Adelaide
4. Burroughs, L. F., *Rep. agric. hort. Res. Stn Univ. Bristol*, 1955, p. 74
5. Radler, F., *Zentbl. Bakt. ParasitKde.*, II, 1966, **120**, 237
6. Peynaud, E., & Domercq, S., *C.r. hebd. Séanc. Acad. Agric. Fr.* 1959, **45**, 355
7. Webb, R. B., & Ingraham, J. L., *Am. J. Enol. Vitic.*, 1960, **11**, 59
8. Fornachon, J. C. M., *Annl. Technol. agric.*, 1963, **12**, (hors-série), 45
9. Ribéreau-Gayon, J., & Peynaud, E., 'Traite d'Oenologie II', 1961 (Béranger: Paris) p. 497
10. Milisavljevic, D., *Bull. Off. int. Vin.*, 1964, **37**, 374
11. Rankine, B. C., *J. Sci. Fd Agric.* 1967, **18**, 583
12. Kulka, Dora, *J. Inst. Brew.*, 1953, **59**, 285
13. Weinfurtner, F., Eschenbecher, F., & Borges, W., *Zentbl. Bakt. ParasitKde* II, 1961, **114**, 285

NUTRITIONAL VALUE OF MIDDLE EASTERN FOODSTUFFS IV.*—Amino acid composition

By J. JAMALIAN and P. L. PELLETT

The amino acid composition of twenty-six food items common in the Middle East has been determined by ion-exchange chromatography. A pre-oxidation procedure was used for the determination of cystine but was not found satisfactory for the determination of methionine. In addition to the amino acid composition the protein quality scores have been calculated by two methods and are reported together with the *E/T* ratios (the total essential amino acids per total nitrogen).

Introduction

The 1965 report of the joint W.H.O./F.A.O. Expert Group upon protein requirements[1] has reconsidered the problem of amino acid patterns in the light of the developments in this field which have occurred since the report of the 1957 F.A.O. committee[2] in which one hypothetical reference protein was described. The pattern recommended by the 1965 committee is based on the pattern of essential amino acids of egg, and the proportion of the limiting amino acid to the total essential amino acids in a protein or protein mixture is compared with the proportion in egg for that particular amino acid. A further recommendation was that the *E/T* ratio (the total essential amino acids per total nitrogen) should also be reported for proteins or protein mixtures. Both these recommendations require accurate determinations for the eight essential amino acids plus cystine and tyrosine. It is no longer enough to determine the level of the expected limiting amino acid, be it tryptophan, lysine or total sulphur amino acids, and to compare this value with the value in the hypothetical reference protein.

A great deal of information now exists in the literature upon the amino acid composition of foods, and the compilation of Orr & Watt[3] is perhaps the best known. A more recent publication is that of the Nutrition Division of F.A.O.[4] However, relatively few data, at least in the detail required for the 1965 scoring procedure, exist upon many of the food items commonly consumed in the Middle East. This present work was initiated to complete this omission.

No attempt has been made to be exhaustive in the selection of food items for analysis; the main criterion has been whether the particular item can in fact supply sufficient protein to the consumer's daily intake to make the con-

* Part III: *J. Sci. Fd Agric.*, 1967, **18**, 227

sideration of its amino acid composition meaningful. Thus both low-protein sources, which are consumed in considerable quantities, and high-protein sources, which may only be taken infrequently and in small amounts, have been included.

The analysis of amino acids using column chromatography techniques[5] has now been developed into a specialised automatic technique using expensive equipment;[6] the accuracy of such analysis is high. However, for the determination of the amino acid composition of proteins, a protein or food item must be cleaved into its component amino acids. Unfortunately the composition of an amino acid mixture can be determined with greater precision than a protein hydrolysate representative of the composition of the protein can be produced.[7] This applies to the hydrolysis of pure proteins, and also applies particularly where the protein is associated with high levels of carbohydrates, fats, minerals and other components which can often seriously increase the destruction of certain amino acids during hydrolysis.

Both enzymic and acid hydrolyses have been used, each procedure having certain advantages. The enzymic procedure gives less destruction of amino acids than the acid hydrolysis and also more nearly simulates digestion by the body; however, the hydrolysis is frequently incomplete and the results are complicated by the presence of peptides. Acid hydrolysis is complete but certain amino acids are destroyed to varying degrees during the process. As a result, examination of acid hydrolysates prepared by heating for 20, 40 and 70 hours has been recommended for an accurate picture of the amino acid composition of a protein, by extrapolation to zero time. For foodstuffs containing excess carbohydrate a recommended procedure is acid hydrolysis in the presence of a large excess of acid,[8] and this procedure has been followed in these investigations. A full description of various hydrolytic procedures for proteins prior to amino acid analysis has been given by Kimmel.[7]

Experimental

Hydrolysis

Reasonably large samples (~200 g) of the edible portion of the foodstuff to be analysed were (after freeze-drying, if necessary) ground to a fine powder in an electric mill. From a knowledge of the approximate protein content, a sample containing ~12 mg of nitrogen was accurately weighed for hydrolysis. A duplicate sample was weighed at the same time for total nitrogen analysis (semi-micro Kjeldahl procedure) so that possible errors caused by changes in moisture content were eliminated. Hydrolysis was performed under reflux for 24 hours using 300 ml of 6 N-HCl with washed nitrogen gas being slowly bubbled through the sample. The hydrolysate was filtered through a sintered glass disc to remove humin, a small amount of distilled water being used for washing, and then it was concentrated to dryness in a rotary evaporator. The dried residue was re-dissolved in distilled water and again evaporated to dryness twice, before filtration through a Whatman No. 4 paper to remove precipitated humin; it was dissolved in a pH 2·2 citrate buffer (0·20 N with respect to sodium) and the solution was made up to 25 ml.

Sulphur amino acid analysis

Cystine and methionine are partly destroyed during normal hydrolysis, but a pre-oxidation procedure converting cystine to cysteic acid has been described to mininise this destruction.[9] Bujard (personal communication) has slightly modified this procedure so that both cystine and methionine are measured in their oxidised forms as cysteic acid and methionine sulphone respectively. A sample containing ~7 mg N was treated with 25 ml performic acid, freshly prepared by mixing 72 ml of 98% formic acid with 1·5 ml methanol and 7·5 ml 30% H_2O_2 which was allowed to stand for 2 hours at room temperature and was then cooled before use. After overnight oxidation at 4° in a refrigerator the suspension was diluted with 25 ml of distilled water, treated with 2 ml of 48% HBr, and evaporated to dryness in a rotary evaporator at 40°. This was then hydrolysed with 100 ml of 6 N-HCl for 24 h.

Tryptophan analysis

Because of the total destruction of tryptophan during acid hydrolysis for 24 h a chemical method based on that of Lombard & De Lange[10] was used. Duplicate samples (~600 mg) were weighed into soft glass screw-capped bottles. 25 ml of 0·05 N-NaOH, 10 ml of enzyme solution (2 g papain in 100 ml distilled water, shaken, and then filtered before use) and 10 drops of 5% NaCN solution were then added to each bottle. For the tryptophan content of the papain, a 30 ml sample was treated with 25 ml of the NaOH and 10 drops of the NaCN. All containers were sealed and digested overnight in a water bath at 70 ± 1°C. After cooling, the samples were made up to volume (90 ml), mixed and allowed to settle. To 5 ml of each hydrolysate in a 125 ml glass-stoppered conical flask were added 5 ml of 0·1 N-KOH and 3 ml of CCl_4, the flasks were shaken for 10–20 minutes and some of the upper layer was centrifuged at 3000 rev/min. One ml of the clear supernatant fluid was pipetted into each of three test tubes, one of which was the blank, followed by one ml of *p*-dimethylamino benzaldehyde (5% in conc. HCl) (one ml of distilled H_2O into the blank). Five ml conc. HCl were added to all tubes which were then shaken, and after 10 minutes 2 drops of a freshly prepared 0·2% $NaNO_2$ solution were added and the whole was mixed for 5 seconds and allowed to stand for 5 minutes for colour development. Colour intensities (stable for 1 h) were read at 590 nm against the blank on a spectrophotometer (Spectronic 20). The optical densities were interpreted by comparing with the similarly obtained values for graded quantities of standard tryptophan solutions (0 μg to 75 μg). The standard curve procedure commenced at the point in the main procedure where one ml quantities were pipetted into the three test tubes. Tryptophan content of the original material was thus calculated with due adjustment being made for the tryptophan content of the enzyme.

Chromatography

Suitable aliquots of the hydrolysates, usually 0·5–1 ml, were analysed on an automatic amino acid analyser (Phoenix, Model K-8000) based on the method devised by Spackman, Stein & Moore.[6] The basic amino acids (lysine, histidine, ammonia, and arginine) were separated on a short (15 cm) column, while the acidic and neutral amino acids (cysteic acid, methionine sulphoxides, aspartic acid, methionine sulphone, threonine, serine, glutamic acid, proline, glycine, alanine, cystine, valine, methionine, isoleucine, leucine, tyrosine and phenylalanine) were separated on a long (150 cm) column. The peak areas were compared with those obtained with standard quantities of pure L-amino acids. Cysteic acid, if present, was taken with cystine, methionine sulphoxides and methionine sulphone were taken with methionine, and alloisoleucine with isoleucine.

The pre-oxidised hydrolysates were analysed in a similar manner using only the 150 cm column. For the sulphur amino acids, a 5½ h analysis time was used initially, which is enough to allow the emergence of cysteic acid and methionine sulphone. Later, due to apparent low recoveries of methionine after oxidation, the elution time was increased to 12 h (i.e. after the normal emergence of methionine) and it was observed that there was always some unoxidised methionine present.

Results and Discussion

The contents of amino acids per g nitrogen were calculated from peak areas, and the values obtained were transferred to punched cards. A programme for the IBM 1620 computer was used which calculated the total nitrogen recovery, the E/T ratios and the scores on the basis of the four potentially limiting essential amino acids using both the 1957 reference pattern and the 1965 scoring procedure.

One μmole of nor-leucine was added with the sample to test recovery, a correction factor was applied, assuming 100% nor-leucine recovery. However, if the discrepancy was large, no correction factor was applied, and the sample was re-run. Samples were also re-run when calculation showed poor nitrogen recovery.

The results obtained are shown in Table I. As was discussed earlier, the sulphur amino acids are amongst the most labile and are also the most likely to be limiting in

TABLE I

Amino acid composition and protein quality scores for selected Middle Eastern foodstuffs

	Amino acids mg/g N																				Score *			Tot. N
						S-containing																		
	Try	Thr	Ile	Leu	Lys	Met	Cys	Total	Phe	Tyr	Val	Arg	His	Ala	Asp	Glu	Gly	Pro	Ser	NH_3	1957	1965	E/T	(g)
Milk, milk products and egg																								
Casein	83	273	355	634	524	162	21	183	344	362	478	254	187	204	471	1312	125	979	425	137	68 (S)	53 (S)	3·24	1·02
Akkawi cheese	91	222	347	647	476	168	33	201	341	362	468	245	201	188	455	1281	128	79	441	144	74 (S)	59 (S)	3·16	0·99
Arishé cheese	89	260	343	656	538	210	38	248	357	358	506	218	194	215	469	1314	125	780	311	178	91 (S)	69 (S)	3·36	1·02
Mountain cheese	74	281	306	638	522	193	34	227	285	276	519	211	197	138	387	1355	95	915	296	167	82 (Tr)	67 (S)	3·13	0·98
Shanklish cheese	106	266	359	704	674	209	46	255	365	385	482	249	196	263	502	1285	140	810	329	160	94 (S)	66 (S)	3·60	1·04
Labne	83	294	384	723	545	197	50	247	379	395	504	240	178	261	565	1478	151	800	392	146	92 (S)	64 (S)	3·55	1·04
Egg	96	290	358	584	455	213	155	368	361	335	482	454	176	382	626	816	199	203	450	126	107 (Tr)	88 (Th)	3·33	1·00
Meat and meat products																								
Lungs (Sheep)	64	245	198	530	424	109	98	207	291	206	387	384	171	439	538	719	607	478	278	123	71 (Tr)	75 (S)	2·55	0·97
Spleen (Sheep)	69	255	396	556	484	119	80	199	284	182	408	395	208	404	542	728	406	348	273	120	74 (S)	65 (S)	2·83	0·96
Brain (Sheep)	60	327	243	544	359	155	96	251	320	241	425	337	150	377	665	990	348	381	231	231	67 (Tr)	70 (Tr)	2·77	1·04
Legumes																								
Chickpea (*Cicer arietinum*	86	248	296	501	463	91	93	184	488	212	331	537	168	271	751	1028	179	330	319	125	68 (S)	61 (S)	2·81	0·98
Fenugreek (*Trigonella foenum-graecum*)	45	201	298	409	357	82	100	182	237	191	237	646	144	225	622	967	269	301	279	116	50 (Tr)	67 (Tr)	2·16	0·91
Lentils (*Lens esculenta*)	36	266	282	485	484	60	60	120	327	211	353	555	200	268	716	1007	262	282	321	100	40 (Tr)	44 (S)	2·52	0·95
Sweet lupin (*Lupinus albus*)	42	237	295	472	304	41	97	138	252	299	308	679	138	238	682	1328	270	298	325	157	47 (Tr)	55 (S)	2 35	0·95
Groundnut meal (Av. 5 samples) (*Arachis hypogaea*)	55	170	198	388	212	54	69	123	291	264	268	689	143	233	695	1106	350	285	239	129	47 (S)	60 (S)	1·97	0·93
Soyabean (Av. 3 samples) (*Glycine soja*)	82	255	313	537	450	87	104	191	352	273	346	514	187	284	757	1156	275	408	336	123	71 (S)	63 (S)	2·80	1·02
Broad beans, fresh (*Vicia faba*)	33	159	222	389	338	33	38	71	215	153	374	760	126	255	659	781	201	206	240	202	26 (S)	34 (S)	1·95	0·95
Nuts																								
Dry almonds (*Prunus amygdalus*)	35	152	216	391	140	44	65	109	301	183	325	610	139	242	589	1366	326	303	219	202	39 (Tr)	55 (S)	1·85	0·96
Green almonds (*Prunus amygdalus*)	25	179	177	309	249	62	64	126	207	136	229	218	93	254	1560	435	192	277	295	295	28 (Tr)	49 (Tr)	1·64	0·88
Pistachio nut (*Pistacio vera*)	58	172	247	427	303	103	101	204	305	187	377	521	132	263	541	1245	283	251	346	141	64 (Tr)	77 (Th)	2·28	0·92
Watermelon seeds (*Citrullus vulgaris*)	73	210	308	438	162	161	70	231	496	195	335	962	149	313	539	1092	340	271	277	108	60 (L)	53 (L)	2·45	1·04
Pine seeds (*Pinus pinea*)	47	122	179	357	156	93	138	231	176	208	249	1082	125	246	447	570	265	279	143	91	52 (Tr)	72 (Th)	1·73	0·89
Grains and their products																								
Burghul (Av. 2 samples)	45	172	195	390	160	90	123	213	253	183	230	270	120	222	290	1397	241	637	260	197	50 (Tr)	68 (L)	1·89	0·85
Local flour	45	152	206	400	127	107	138	245	293	202	271	230	115	173	148	1839	192	727	264	254	47 (L)	52 (L)	1·94	0·90
Arabic bread	38	166	203	461	133	99	138	237	390	214	288	264	149	197	274	2127	209	834	239	283	42 (Tr)	50 (L)	2·11	1·02
Miscellaneous																								
Laubina 104	50	198	241	442	287	105	93	198	289	201	315	320	132	220	460	1293	205	526	291	162	56 (Tr)	69 (Tr)	2·33	0·91
Laubina 105	42	203	252	459	256	96	107	203	332	209	320	387	146	247	489	1508	135	570	287	225	47 (Tr)	59 (Tr)	2·28	0·97
Kishk	61	205	272	512	324	121	107	228	314	261	326	313	167	235	375	1563	217	745	299	228	68 (Tr)	78 (Tr)	2·50	1·01

* Letter in brackets indicates the limiting amino acid: Tr = Tryptophan, Th = Threonine, L = Lysine and S = total sulphur amino acids
Akkawi Cheese .....Medium hard cheese from whole cows milk
Mountain Cheese ....Medium hard cheese from whole goats milk
Arishé Cheese .....Medium hard cheese from acidified whey remaining from mountain cheese preparation
Shanklish Cheese.....Prepared from fermented de-fatted goats milk yoghurt, which is then covered with thyme, sumak and seasame seeds
Burghul ..... Parboiled wheat
Laubina 104 & 105 Infant food preparation from American University of Beirut;[11] contains burghul and chickpea
Kishk .....Burghul and yoghurt[12]

human diet;[13] thus special procedures have been advocated for their analysis. All samples were analysed by a pre-oxidation procedure as described earlier in addition to the normal direct hydrolysis procedure.

Pre-oxidation converted cystine to cysteic acid and methionine to methionine sulphone, and these were separated and measured by the subsequent ion-exchange chromatography. The elution times at 30 ml/h flow rate for the various sulphur amino acids were approximately 110 minutes for cysteic acid, 230 minutes for methionine sulphoxides, 280 minutes for methionine sulphone, 600 minutes for cystine and 690 minutes for methionine.

Normally, elution is stopped after the emergence of methionine sulphone whether special elution techniques are used[14,15] or whether the normal chromatography procedure is followed. Low recoveries of methionine were noted compared to the values obtained when the normal hydrolysis procedure was followed, and it was considered that perhaps some methionine was remaining unoxidised. By eluting the oxidised samples for 12 hours it was observed that there was indeed a small quantity of residual methionine which had resisted oxidation. If the oxidation procedure had been at fault, or there had been for example insufficient penetration of the oxidising reagent to the sample, there might have been residual unoxidised cystine present. However, in no oxidised sample was there any free cystine remaining, and complete oxidation to cysteic acid always took place. For eleven of the above samples elution was continued until after the emergence of methionine; in Table II is shown the distribution of the sulphur amino acids obtained. There was in all cases a small residue of unoxidised methionine which, was not enough to prevent a lower value for total methionine after oxidation than that obtained by normal hydrolysis. Cystine showed either no change or a net increase caused by the oxidation procedure.

Additional analyses have recently been performed upon mixed diets. These are not reported in Table I, but, the mean values obtained for both mixed diets and the individual food items are shown in Table III.

It will be seen that there was a net increase either in absolute or percentage terms in the cystine content following the oxidation procedure, but that there was in general a net loss in methionine whether the elution was continued for 330 minutes (i.e., methionine sulphone eluted) or for 700 minutes (residual methionine eluted), the loss being lower after the 700 minutes elution because of the small quantity of residual methionine present. The apparent discrepancy between an average loss in absolute terms and a gain in percentage terms is explainable by the presence of one sample which showed a small increase in methionine following pre-oxidation which,

TABLE II

Effect of pre-oxidation on the cystine and methionine content of some Middle Eastern food items

	Sulphur amino acids (mg/g N)							
	Cystine		Methionine					
			Pre-oxidised			Normal hydrolysis		
	Pre-oxidised	Normal hydrolysis	As methionine sulphone	Methionine	Total methionine	As methionine sulphoxides	Methionine	Total methionine
Casein	21	0	171	3	174	25	137	162
Akkawi Cheese	33	29	118	9	127	16	152	168
Mountain Cheese	34	16	110	5	115	21	172	193
Arishé Cheese	38	35	116	2	118	30	180	210
Dry Almonds	65	67	33	1	34	31	13	44
Fresh broad beans	38	30	32	1	33	18	0	18
Brain	96	86	116	3	119	47	108	155
Soyabean	104	90	98	1	99	19	68	87
Groundnut (1)	73	58	59	8	67	0	64	64
,, (2)	79	57	63	9	72	33	25	58
,, (3)	62	57	46	5	51	16	40	56

TABLE III

Effect of oxidation and time of elution upon the mean recovery of cystine and methionine

Amino acid	No. of samples	Average increase or decrease in amino acid content over unoxidised samples	
		Absolute* mg/g N	%
Cystine	25	9·4 ± 2·5	13·0
Methionine (330 min)	19	−13·4 ± 4·6	−11·0
Methionine (700 min)	14	− 4·9 ± 8·1	+ 4·6

Cystine measured as cysteic acid, methionine measured as methionine sulphone (330 min) and methionine sulphone plus methionine (700 min)

* ± Standard error of the mean

however, was very large as a percentage increase.

Because of the variability in methionine recovery following oxidation and the time needed to elute the residual unoxidised methionine, the following procedure was adopted for the results shown in Table I. The oxidation procedure was considered to be valid for cystine only; thus the values for cystine are those obtained after pre-oxidation. The methionine values are (with one exception) the values obtained by normal hydrolysis and the values for total sulphur amino acids are the sum of the two values. In the case of fresh broad beans the oxidised value was taken for the methionine because it was much higher than that obtained by normal hydrolysis and because the value obtained following normal hydrolysis showed an abnormality in that the whole of measured methionine was present as methionine sulphoxide indicating perhaps that the methionine was linked in the protein in such a way that it was especially susceptible to hydrolysis.

In addition to amino acid composition, and total N recovery, the protein quality 'scores' have been calculated either on the basis of the 1957 report using the values of 90, 180, 270 and 270 μg/g N as the levels for tryptophan, threonine, lysine and total sulphur amino acids respectively in F.A.O. reference protein, or on the basis of the 1965 report where comparison is made against the level of total essential amino acids present (Table I). The two ratios described by the 1965 committee were termed the A/E ratio and the E/T ratio, the former being the proportion each essential amino acid contributes to the total essential amino acids, the score being derived from this by comparison with the A/E ratio for egg; the E/T ratio was described as the ratio of essential amino acids to total nitrogen expressed as g of essential amino acids per g total nitrogen. 'Scores' are normally only of value for mixed diets; it is, however, useful to give these values together with the amino acid data since the limiting amino acid is specified and it is easier to visualise its degree of deficiency. The E/T ratios agree very well with the values given in the 1965 report for a series of selected foodstuffs. The high values for the various cheese products and the low values for immature green almonds, pine seeds and burghul are noteworthy.

There are differences between the scoring procedures; in only 18 of the 28 cases, do the two procedures indicate the same amino acid as being the one limiting. However, the value for the second limiting amino acid is always close to that of the first when this discrepancy occurs. The difference between the two procedures of scoring seems to be greatest when the E/T ratio is high, as in the cheese samples.

Amongst the individual values the low level of tryptophan in both green and dry almonds, broad beans, lentils and burghul should be noted. A cereal–legume mixture based upon lentil and burghul would always be low in tryptophan and a protein food high in tryptophan, such as in chickpea or milk, would need to be incorporated in order to produce a mixture of high protein value. Materials high in methionine or total sulphur amino acids are of importance because most world diets are limiting in sulphur amino acids.[11] Other than egg, the importance of which as a methionine source is well documented, the cheeses, brain, watermelon seeds, pine seeds and the wheat flour products are high in methionine. Good sources of lysine to supplement the lack of lysine in wheat flour products are the cheeses and milk products, chickpea, lentil, soyabean and spleen.

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Department of Food Technology and Nutrition
Faculty of Agricultural Sciences
American University of Beirut,
Beirut, The Republic of Lebanon

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References

1. FAO/WHO 'Protein requirements', *FAO Nutr. Mtgs Rep. Ser. No. 37/WHO Tech. Rep. Ser. No. 301*, FAO, Rome, 1965
2. FAO 'Protein requirements', *FAO Nutr. Stud. No. 16*, FAO, Rome, 1957
3. Orr, M. A., & Watt, B. K., 'Amino acid content of foods', Home Economics Research Report No. 4, Washington, D.C., 1957
4. FAO 'Amino acid content of foods', Nutrition Division, FAO, Rome, 1963
5. Moore, S., Spackman, D. H., & Stein, W. H., *Analyt Chem.*, 1958, **30**, 1185
6. Spackman, D. H., Stein, W. H., & Moore, S., *Analyt Chem.*, 1958, **30**, 1190
7. Kimmel, J. R., 'Some recent advances in techniques for protein degradation', Chapter 8 of Symposium on Foods: Proteins and their Reactions (Ed. Schultz, H. W., & Anaglemier, A. F.) (Avi Publishing Co., Westport, Connecticut) 1964
8. NAS/NRC, 'Evaluation of protein quality', National Academy of Sciences, National Research Council Public. No. 1100, Washington, D.C., 1963
9. Moore, S., *J. biol. Chem.*, 1963, **238**, 235
10. Lombard, J. H., & D. J. DeLange, *Analyt Biochem.*, 1965, **10**, 260
11. Tannous, R. I., Cowan, J. W., Rinnu, F., Asfour, R. J., & Sabry, Z. I., *Am. J. Clin. Nutr.*, 1965, **17**, 143
12. Frankul, W., & Pellett, P. L., *Proc. Nutr. Soc.*, 1959, 18, xxxvi
13. Rose, W. C., Haines, W. J., & Warner, D. T., *J. biol. Chem.*, 1954, **206**, 421
14. Bujard, E., & Mauron, J., in NAS-NRC Public. No. 1100, 1963
15. Lewis, O. A. M., *Nature, Lond.*, 1966, **209**, 1239

IDENTIFICATION OF SOME CONSTITUENTS OF CARROT SEED OIL

By R. M. SEIFERT, R. G. BUTTERY and L. LING

The composition of carrot seed oil has been studied using gas–liquid chromatography separation of components and characterisation by infra-red absorption and mass spectrometry. Evidence was found for the identities of 23 components. Of these, camphene, α-terpinene, terpinene-4-ol, α-terpineol, bornyl acetate, γ-decanolactone, β-selinene, α-gurjunene and coumarin had not been previously reported as constituents of carrot seed oil.

Introduction

The composition of the essential oil from seeds of *Daucus carota* has been of interest to many investigators. Some of the compounds reported in carrot seed oil are: formic acid,[1] acetic acid as an ester,[1] butyric acid,[1] palmitic acid,[1,15] asarone (2,4,5-trimethoxy propenyl benzene),[1,6,8,11,12] asaronaldehyde (2,4,5-trimethoxy benzaldehyde),[2,18] petroselinic acid,[15] oleic acid,[15] linoleic acid,[15] p-thymol,[6] L-α-pinene,[1,6,8,10,11,12,19] β-pinene,[6,12,19] L-limonene,[1,6,11,19] L-sabinene,[6,7,9,12] no-pinene,[7,9] myrcene,[8,10] a $C_{10}H_{16}$ terpene,[9] geraniol,[3,7,11,19] geranyl acetate,[6,7,9,12,17,19] linalool,[6,12] ionene,[12] citronellol,[3] citral aldehyde,[3] carotol,[1,5,6,8,11,16,17,19] β-bisabolene,[1,6,8,10,11,12,19] epoxydihydrocaryophyllene,[17] daucol,[1,5,6,11,19] a bicyclic unsaturated sesquiterpene $C_{15}H_{24}$ b.p. 110–112° azulene type,[7,9] a $C_{30}H_{62}$ saturated compound m.p. 67–67·5°,[7,9,12] a $C_{15}H_{28}O$ compound m.p. 120°,[7,9] bergamotene,[6,8,10,12,19] a bicyclic sesquiterpene $C_{15}H_{26}O$ m.p. 120° azulene type,[12] caryophyllene,[3,19] daucine,[6,11] α-curcumene,[6] elemencin,[6] and a sesquiterpene alcohol of selinane type.[13]

The composition of carrot seed oil varies from variety to variety not only qualitatively but quantitatively.[12,16] Differences in reported composition reflect not only varietal differences, but also changes occurring in the oil due to different methods of isolation and identification of these compounds.

Interest in the aroma of fresh carrot root led to a preliminary investigation of carrot seed oil from *Daucus carota* L. obtained from Fritzsche Brothers, Inc., New York.

It was hoped that a study of carrot seed oil would lead to a component or group of components with characteristic fresh carrot aroma. This knowledge would be of considerable interest in controlling the flavour of the various processed forms of carrots.

Experimental

Materials

Carrot seed oil was obtained from Fritzsche Brothers, Inc.* It is an oil produced in France by the steam distillation of the seed of *Daucus carota* L.

Authentic samples of organic compounds were obtained from reliable commercial sources or synthesised by well established methods.

* Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

Preliminary fractionation of carrot seed oil

Two main approaches to the analysis of the components of carrot seed oil were used. The first of these used fractional distillation under vacuum to divide the oil into 6 fractions: b.p. 30–62°, 12 mm, 21% of the oil; b.p. 62–99°, 12 mm, 11%; b.p. 99–123°, 12 mm, 23%; b.p. 123–140°, 12 mm, 17·9%; b.p. 58–107°, 0·07 mm, 21·4%; Residue 5·1%. Only the two lower b.p. fractions were analysed by g.l.c.

The second approach involved vacuum distillation of the whole oil to remove non-volatile material. This gave 86% of the oil, b.p. 25–112° at 0·08 mm Hg. The distilled oil was separated into its hydrocarbon and oxygenated fractions by the following procedure. A sample of the oil (25 g) was placed on a column of silica gel (5 × 24 cm), Fisher 28–200 mesh). The hydrocarbon fraction was eluted with 1500 ml of pentane. The oxygenated fraction was then eluted with 1500 ml of ethyl ether. The solvents were removed on a rotary evaporator. The hydrocarbon and oxygenated fractions were then analysed separately by g.l.c.

Gas chromatography

The two fractions of lower b.p. containing oxygenated and hydrocarbon material were subjected to g.l.c. Approximately 50 components were isolated and examined. G.l.c. separation was made initially on a 20 ft long by ⅜ in. o.d. aluminium column packed with 30% Carbowax 20M on 30–60 mesh Chromosorb P (acid-washed). Further fractionation was made on a 20 ft by ⅜ in. o.d. alumium column packed with 30% Apiezon M on 30–60 mesh Chromosorb P (acid-washed), and a 10 ft long by ¼ in. o.d. aluminium column packed with 10% SF96(350) with Igepal CO-880 (1% of the silicone) on 80–100 mesh Chromosorb P.

The hydrocarbon and oxygenated fractions from the silica gel separation of the whole distilled oil were examined by g.l.c. The hydrocarbon fraction was resolved into its components using the 20 ft by ⅜ in. o.d. aluminium column packed with 30% Carbowax 20M on 30–60 mesh Chromosorb P (acid-washed), and programming non-linearly from 168–190°. Twenty-one fractions were collected and examined by i.r. spectroscopy, and in some cases mass spectroscopy.

The oxygenated fraction was first resolved into 24 separated peaks using the 20 ft by ⅜ in. o.d. Carbowax 20M column and programming non-linearly from 180–210°C. Each of the peaks separated from the Carbowax column was then further resolved by g.l.c. separation on a 6 ft long by ¼ in. o.d. aluminium column packed with 10% Apiezon M with Igepal CO-880 (5% of the Apiezon) on 80–100 mesh Chromo-

sorb P. This gave over 40 separated peaks altogether which were all examined by infra-red spectrometry and in some cases mass spectrometry.

G.l.c. of the whole oil compared with g.l.c. of the oil with known compounds was run on a capillary column. The capillary column was 150 ft by 0·01 in. i.d. stainless steel coated with silicone SF96(100) and Igepal CO-880 (5% by weight of the silicone).

Injectors for all columns were stainless steel and were heated at 200°. A split-stream injector with a split of 1/300 was used for the capillary column. Detectors were thermal conductivity for the packed columns and flame ionisation for the capillary.

Infra-red (i.r.) absorption spectra

I.r. spectra were run on a Perkin Elmer 237 double beam grating instrument. The untreated samples were generally run as films using 3 × 9 mm salt plates or in CCl_4 solution in a 0·05 mm microcell.

Mass spectra (m.s.)

Mass spectra were run using a modified CEC model 21–620 mass spectrometer.

Results and Discussion

The second approach using silica gel chromatography for preliminary fractionation was in general a more satisfactory approach than fractional distillation of the oil. However, fractional distillation was advantageous in confirming identities because adsorbents used in silica gel chromatography can occasionally cause misleading rearrangements. In the present work, it was found that there was some rearrangement of the sabinene on the silica gel.

All peaks separated by g.l.c. were analysed by i.r. absorption spectroscopy. Twenty-three of these peaks were identified. Their spectra were consistent with that of authentic samples. I.r. spectra found are reported in microns with the size of the maxima abbreviated as VS meaning very strong, S meaning strong, M for medium, W for weak, and VW for very weak. The spectra found for the compounds isolated from the carrot seed and not previously reported in carrot seed oil are listed below.

Camphene: VS (11·3), M (6·05, 6·8, 6·9, 7·35, 8·9, 9·1), W (6·0, 6·7, 7·0, 7·2, 7·7, 7·8).

α-Terpinene: VS (12·2), S (6·1, 6·8, 6·9, 7·0, 7·3, 7·4), M (7·6, 7·8, 8·1, 8·7, 9·8, 10·5, 11·4), W (6·25, 7·7, 8·0, 9·3, 9·4, 9·5, 9·6, 10·2, 10·9, 12·8, 13·0, 13·4, 13·8, 14·7, 14·9).

α-Gurjune: VS (6·9, 7·25), S (7·2, 7·35, 8·9, 9·4, 10·7, 12·7), M (6·0, 8·0, 8·35, 8·7, 9·1, 9·8, 10·2, 10·5, 13, 13·5), W (7·0, 7·4, 7·45, 7·5, 7·6, 7·7, 7·9, 8·3, 9·2, 9·6, 10, 11·25, 11·5, 12, 13, 13·3, 14, 14·8).

β-Selinane: VS (11·3), S (6·1, 6·9, 6·95, 7·2), W (5·6, 7·1, 7·9, 8·1, 8·5, 8·6, 8·7, 9·2, 9·6, 10·2, 10·5, 10·7, 10·8, 11·7, 12·0, 12·5, 13·0, 14·0).

Terpinene-4-ol: S (9·35, 11·3), M (6·7, 6·9, 6·95, 7·25, 7·3, 9·75, 10·0, 10·8, 11·6, 12·5), W (7·7, 8·0, 8·2, 8·5, 8·6, 8·9, 10·5, 13·0, 13·7), VW (9·2, 9·5, 9·9).

Bornyl acetate: VS (5·75, 7·3, 8·0, 9·65), S (6·8, 6·9, 7·2, 9·0, 9·55), M (7·7, 8·6, 8·8), W (6·1, 7·5, 8·4, 9·2, 10·1, 10·2, 10·5, 11·0, 11·3, 11·8, 12·2, 12·7, 13·5), VW (6·1, 10·65, 10·85, 12·0, 15·2, 15·7).

α-Terpineol: S (6·9, 7·25, 7·3, 10·8, 11·0, 11·95, 12·5), M (7·75, 8·2, 8·65, 8·85, 9·6, 9·8, 10·5, 12·8, 12·9), W (8·0, 9·0, 9·3, 9·6, 10·1, 10·2, 13·25, 15·2, 15·8).

γ-Decanolactone: VS (5·6, 8·45), S (6·8, 10·9), M (7·3, 8·8, 9·7), W (7·0, 7·25, 7·8, 8·1, 9·3, 10·25, 11·8, 12·3, 13·7).

Coumarin: VS (5·7, 5·75, 5·8, 6·2, 6·9, 8·9, 9·1, 10·8, 12·0), S (6·15, 6·4, 7·1, 7·85, 7·9, 11·2), M (8·15), W (5·6, 6·0, 6·7, 7·5, 8·3, 10·6, 11·5, 14·6).

G.l.c. retention time on the capillary column, m.s., and i.r. were used to analyse the carrot seed oil. The identities of components found by these methods are listed in Table I. Fig. 1 shows a capillary gas chromatography run of the distilled whole oil. It can be seen that carrot seed oil is very complex but that the major components are sabinene, geranyl acetate and carotol. β-Selinene is the first selinene hydrocarbon to be found in carrot seed oil, although Pigulevskii & Motskus[13] had isolated a sesquiterpenoid alcohol with a selinane ring system. α-Gurjunene which possesses the unusual combination of 3-, 5- and 7-membered rings also had not been detected previously although an azulene type of sesquiterpene had been reported by Pigulevskii *et al.*[12] but not characterised.

Many of the components of the carrot seed oil had particularly potent odours but in general their odours were quite different from that normally associated with the carrot

TABLE I

Identities of components found in carrot seed oil

Peak No. in Fig. 1	Confirmed identity*	Tentative identity
3	α-Pinene	
4	Camphene**	
6	Sabinene, m.s.	
6	β-Pinene, m.s.	
8	Myrcene, m.s.	
11	α-Terpinene**	
12	p-Cymene	
13	Limonene	
17	γ-Terpinene	
22	Linalool	
30	Terpinene-4-ol**	
32	α-Terpineol**	
39	Bornyl acetate**	
40	Geraniol	
45	Geranyl acetate	
47	Caryophyllene	
56	γ-Decanolactone**	
59	β-Selinene**	
59	Coumarin**	
72	Carotol†	
	Duacol†	
	α-Gurjunene**†	
	β-Bisabolene	
		2-Octanone or related compound
		Geranyl formate**
		Trans-6,(7)-2,6-dimethyl-4,6-octadiene**†

* Data identical to that of authentic sample, m.s. = Mass spectrum evidence. All confirmed by i.r. absorption spectrum evidence; all but the last four confirmed by g.l.c. retention time evidence

** Not previously reported in carrot seed oil

† No authentic samples available but spectra consistent with published data[4,14]

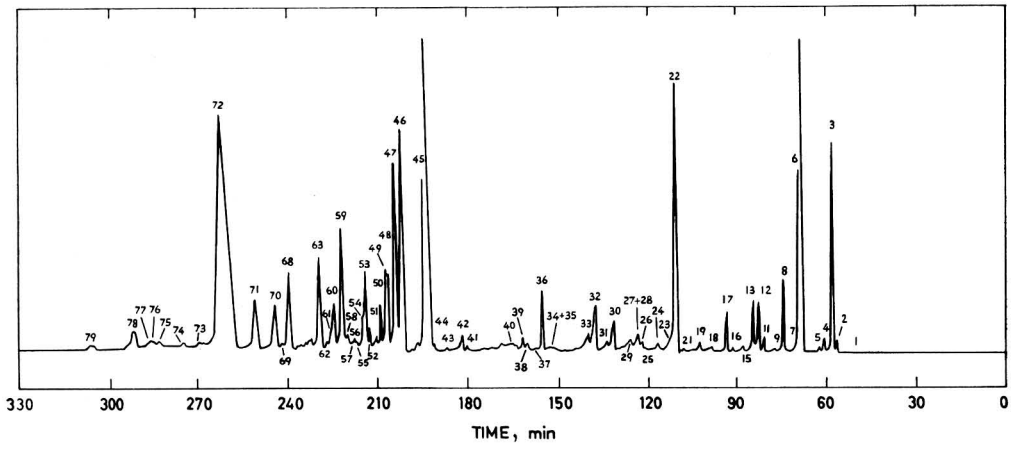

FIG. 1. *Programmed capillary g.l.c. analysis of distilled carrot seed oil using a* 150 *ft* × 0·01 *in. i.d. stainless steel capillary coated with silicone SF*96(100) *and programming from* 50–160° *and holding*

3	α-pinene	— 3%
6	sabinene	— 15%
22	linalool	— 6%
45	geranyl acetate	— 17%
47	caryophyllene	— 4%
59	β-selinene	— 4%
72	carotol	— 18%

root. Sabinene and myrcene were the only components which had a carrot root-like odour. On examination of carrots, these two hydrocarbons were found in unusually high concentration in the stems of the carrot tops. Although there are indications that the composition varies in different parts of the carrot root, the average concentration of these two components in the whole root was not very high.

Western Regional Research Laboratory,
Agricultural Research Service,
U.S. Dept. of Agriculture,
Albany, California 94710

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References

1. Guenther, E., 'The Essential Oils', 1950, **4,** 589 (New York: D. Van Nostrand Co., Inc.), and references therein.
2. Khafagy, S. A., *Svensk Farm Tidskr*, 1963, **67,** 349 (*Chem. Abstr.*, 1962, **59,** 9016)
3. Makarova, G. V., & Borisyuk, Yu., G., *Trudy Khar'kov Farmatsevt Inst.*, 1957, No. 1, 244–9 (*Chem. Abstr.*, 1959, **53,** 15224e)
4. Mitzer, B. M., Theimer, E. T., & Freeman, S. K., *Appl. Spectrosc.*, 1965, **19,** (6), 169
5. Nigam, S. S., & Radhakrishnan, C., *Perfum. essent. Oil Rec.* 1963, **54,** 87
6. Pigulevskii, C. V., *et al., Drug Cosmet. Ind.*, 1965, **97** (5), 749
7. Pigulevskii, C. V., & Kovaleva, V. I., *Trudy Bot. Inst. V. L. Komarova Akad. Nauk SSSR*, 1955, **5,** 7–20 [*Chem. Abstr.*, 1956, **50,** 17340d]
8. *Ibid.*, 1961, Ser. 5, (8), 15–23 [*Chem. Abstr.*, 1962, **56,** 11727]
9. Pigulevskii, C. V., & Kotaleva, V. I., *Zh. Prikl. Khim.*, 1955 **28,** 1355 [*Chem. Abstr.*, 1956, **50,** 5988i]
10. *Ibid.*, 1959, **32,** 2703 [*Chem. Abstr.*, 1960, **54,** 9213b]
11. Pigulevskii, C. V., Motskus, D. V., & Rodina, L. L., *Zh. Prikl. Khim.*, 1962, **35,** 1143 [*Chem. Abstr.*, 1962, **57,** 7396h]
12. Pigulevskii, C. V., Koraleva, V. I., & Motskus, D. V., *Trudy Vses. Sov. Vilnyus Akad Nauk Litovsk., SSR*, 1959, 153–7 (pub. 1960) [*Chem. Abstr.*, 1961, **55,** 15842g]
13. Pigulevskii, C. V., & Motskus, D. V., *Zh. Obschch. Khim.*, 1962, **32,** 2365 [*Chem. Abstr.*, 1962, **58,** 7980d]
14. Pliva, J., Horak, N., Herout, V., & Sorm F., 'Die Terpene. I. Sesquiterpenes', 1960 (Akademie Verlag: Berlin)
15. Prakash, O., Ram, A., & Gupta, J. C., *J. Proc. Oil Technol. Ass. India*, 1957, Kampur **13** (Pt. 1–2), 42 [*Chem. Abstr.*, 1959, 17540h]
16. Sorm, F., & Urbanek, L., *Colln. Czech. chem. Commun.*, 1948, **13,** 49 [*Chem. Abstr.*, 1948, **42,** 7283c]
17. Stahl, E., *Arch. Pharm.*, 1964, 297, 8, 500 [*Chem. Abstr.*, 1964, **61,** 11001d]
18. Starkovsky, N. A., *J. org. Chem.*, 1962, **27,** 3733
19. Zalkow, L. H., Park, M. K., & Ellis, J. W., *Perfum. essent. Oil, Rec.* 1963, **54,** 507

GLYCERIDE STUDIES

VIII.*—The component glycerides of four *Strophanthus* oils containing an unsaturated hydroxy acid

By F. D. GUNSTONE and M. I. QURESHI

The glycerides of four *Strophanthus* oils containing 9-hydroxyoctadec-12-enoic acid (6–15%) have been examined by thin-layer chromatography on silica and on silica—silver nitrate and by lipolysis. The oils show no unusual features in their glyceride composition; the hydroxy acid behaves like linoleic acid in its distribution pattern.

Introduction

The glyceride composition of many seed oils containing only the common saturated acids along with oleic, linoleic, and linolenic acids has now been determined by the newer methods of lipolysis and/or silver ion thin layer chromatography and/or gas liquid chromatography. The results show the general correctness of the 1,3-random 2-random pattern of acyl group distribution for vegetable fats.[1] It remains to be discovered whether seed fats containing the less common acids follow a similar pattern[2,3] and this paper reports the composition of four oils containing an unsaturated hydroxy ester.

Seed fats containing ricinoleic acid,[4] kamlolenic acid,[5] and an epoxy acid (vernolic)[6] have already been reported. The high content (~90%) of ricinoleic acid in castor oil makes it difficult to draw useful conclusions about the distribution of this acid which behaves unusually in ergot oil (not a vegetable oil) where it forms glycerides having four, five, and six acyl groups by acylation of the ricinoleic hydroxyl group.[7] Kamlolenic acid (18-hydroxyeleostearic) also has an acylated hydroxyl group in Kamala oil. It was, therefore, of interest to discover whether the same effect would be observed in *Strophanthus* oils which contain an isomer of ricinoleic acid *viz.* 9-hydroxyoctadec-12-enoic acid,[8] and four different *Strophanthus* oils have been examined.

Experimental and Results

Seeds were crushed in a grinding machine and the oil, completely extracted with petrol (b.p. 40–60°) in a Soxhlet apparatus, was neutralised by quick percolation, in chloroform solution, through a short column of alumina.

The mixed glycerides (and subfractions subsequently obtained) were converted to methyl esters by refluxing with dry methanolic hydrogen chloride.[9] Methyl heptadecanoate required as internal standard was added before transesterification. Ester mixtures were analysed by gas liquid chromatography on a Perkin Elmer fractometer using packed columns with diethylene glycol succinate as stationary phase. Fractions containing hydroxy esters were analysed as trimethylsilyl ethers.[10]

Preliminary tests showed that each *Strophanthus* oil was readily separated by preparative thin layer chromatography into three fractions of differing polarity. The largest and least polar fractions contained none of the hydroxy acid. The second fraction contained one residue of hydroxy acid in each glyceride molecule, and had the same R_f value as the triglyceride fraction in castor oil shown to contain only one ricinoleic acid group. Several small and more polar fractions were also present but were not completely identified: they probably contain mono- and di-glycerides with and without the hydroxy acid. There was no evidence for the presence of triglycerides with two hydroxy acyl groups, or of any triglyceride with more than three acyl groups.

The neutralised oil (100–150 mg) was separated with mixtures of ether and petrol (40 : 60) on ten plates (20 × 20 cm) layered with silica gel (0·3 mm). The bands were made visible with dichlorofluorescein, and three fractions were collected: the least polar glycerides (fraction A), the second fraction (B), and all the more polar components (fraction C). Fractions A and B were purified by repeating this separation process. After recovery each fraction was made up to a 100 ml solution and aliquots withdrawn for further examination. The amount of material in each fraction could be determined by gas liquid chromatography of the derived methyl esters containing a known amount of methyl heptadecanoate as internal standard.[11] Fractions A and B were examined further by silver ion chromatography[11] and by lipolysis.[11] The hydroxy glycerides (fraction B) separated well on silica-silver nitrate when a mixture of ether and benzene (1 : 4) was used as developing solvent. The results are given in Tables I-III.

Discussion

In each oil fraction B was considered to consist of glycerides with one hydroxy acyl group. The content (uncorrected) of hydroxy ester (28–30%) was always lower than expected (33%). When these glycerides were further separated by silver ion chromatography the small fraction travelling furthest up the plate had a much lower content of hydroxy ester and was mainly glyceride without a hydroxy acyl group. All the remaining fractions contained hydroxy ester at a fairly constant level (28·3–33·7, average 30·6) still below the expected value. A correction factor was therefore applied to raise these values to 33·3. It was found that a similar

* Part VII: *Chem. Phys. Lipids*, 1967, **1**, 429

correction was necessary with castor glycerides containing only one ricinoleic acid group.

The unknown nature of the small polar fraction (C) makes it difficult to compare the observed results with those calculated on the basis of any distribution theory. Glyceride composition of each fraction A calculated from the lipolysis results is very similar to the results obtained by silver ion chromatography. Also the lipolysis results for fractions A and B show no unusual features and it is clear that the hydroxy acid behaves very much like linoleic acid and competes very effectively with this acid for the secondary hydroxyl group.

TABLE I

Composition (% mol) of neutralised *Strophanthus* oils and separated glyceride fractions

	Fatty Acids				
	16 : 0	18 : 0	18 : 1	18 : 2	OH–18 : 1
S. sarmentosus					
whole oil	15·1	7·6	45·3	26·2	5·8
fraction A (74·0%)	15·7	7·0	48·6	27·8	0·9
fraction B (19·2%)	14·4	5·8	32·1	16·0	31·7
fraction C (6·8%)	25·6	9·8	41·8	15·2	7·6
S. courmontii					
whole oil*	17·1	7·0	37·2	29·7	8·3
fraction A (71·0%)	16·1	6·3	42·8	34·8	—
fraction B (21·0%)	13·6	4·6	27·5	23·6	30·7
fraction C (8·0%)	20·3	6·6	39·4	22·7	11·0
S. hispidus					
whole oil	13·7	8·4	36·4	27·7	13·8
fraction A (54·1%)	14·3	8·6	45·5	30·2	1·4
fraction B (27·3%)	14·0	5·6	23·9	23·4	33·1
fraction C (18·5%)	14·6	6·2	32·4	27·0	19·8
S. kombe					
whole oil†	12·3	7·8	30·0	32·3	14·6
fraction A (59·4%)	15·5	9·7	35·8	38·0	1·0
fraction B (30·1%)	14·8	6·6	22·2	25·1	31·3
fraction C (10·5%)	20·6	9·1	27·0	25·2	18·1

* Also 16 : 1, 0·7% † also 20 : 0, 3·0%

TABLE II

Component glycerides (% mol) derived from silver ion chromatography of fractions A and B

	S. sarmentosus	*S. courmontii*	*S. hispidus*	*S. kombe*
A fractions				
222*	1·1	2·3	1·4	2·5
221	7·1	8·5	6·5	10·2
220	5·2	8·3	5·4	10·2
211	11·7	11·9	8·5	7·5
210	15·8	15·1	12·4	12·0
111	10·7	8·3	5·9	3·9
200	5·2	5·1	3·8	5·0
110	12·2	7·9	4·3	3·8
100	5·7	3·5	5·8	5·1
000	0	1·3	0·5	0·1
total	(74·7)	(72·2)	(54·5)	(60·3)
B fractions				
H22	0·4	1·4	2·8	3·5
H21	4·2	4·5	6·0	7·0
H20	4·1	5·2	7·0	8·9
H11	3·7	3·1	3·8	2·3
H10	4·7	4·9	5·6	5·8
H00	1·4	0·7	1·8	1·7
total	(18·5)	(19·8)	(27·0)	(29·2)
C fractions				
unidentified	6·8	8·0	18·5	10·5

* These symbols indicate the three acyl groups present in the trigylceride; all isomers are included.
0 = saturated, 1 = monoethenoid, 2 = diethenoid, H = hydroxy acyl group

TABLE III

Lipolysis results

	fraction A			fraction B			
	sat	18 : 1	18 : 2	sat	18 : 1	18 : 2	OH–18 : 1
S. sarmentosus							
triglyceride	22·6	48·5	28·9	20·3	32·0	16·0	31·7
2-monoglyceride	0·9	55·7	43·4	2·6	28·3	24·5	44·6
enrichment factor*	—	1·15	1·51	—	0·88	1·53	1·41
selectivity factor*	—	0·90	1·18	—	0·72	1·25	1·16
S. courmontii							
triglyceride	22·7	42·4	34·9	18·2	27·5	23·6	30·7
2-monoglyceride	1·1	44·1	54·8	1·0	23·7	34·6	40·7
enrichment factor*	—	1·04	1·57	—	0·86	1·47	1·33
selectivity factor*	—	0·81	1·23	—	0·71	1·21	1·10
S. hispidus							
triglyceride	24·3	45·1	30·6	19·6	23·9	23·4	33·1
2-monoglyceride	2·5	38·0	59·5	2·5	20·3	26·2	51·0
enrichment factor*	—	0·84	1·94	—	0·85	1·12	1·54
selectivity factor*	—	0·65	1·50	—	0·70	0·93	1·27
S. kombe							
triglyceride	23·7	37·1	39·2	22·4	23·3	27·0	27·3
2-monoglyceride	2·2	48·7	49·1	0	16·9	33·1	50·0
enrichment factor*	—	1·31	1·26	—	0·73	1·23	1·83
selectivity factor*	—	1·02	0·98	—	0·57	0·95	1·42

* see reference (2) for discussion of these terms

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Chemistry Department,
The University,
St. Andrews, Fife

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References

1. Gunstone, F. D., 'An introduction to the chemistry and biochemistry of fatty acids and their glycerides', 2nd edition, 1967 (Chapman and Hall: London)
2. Gunstone, F. D., Hamilton, R. J., Padley, F. B., & Qureshi, M. I., *J. Am. Oil Chem. Soc.*, 1965, **42,** 965
3. Subbaram, M. R., Chakraberty, M. M., Youngs, C. G., & Craig, B. M., *J. Am. Oil Chem. Soc.*, 1964, **41,** 691
4. Achaya, K. T., Craig, B. M., & Youngs, C. G., *J. Am. Oil Chem. Soc.*, 1964, **41,** 783
5. Achaya, K. T., & Aggarwal, J. S., *Chemy. Ind.*, 1962, p. 1616
6. Tallent, W. H., Cope, D. G., Hagemann, J. W., Earle, F. R., & Wolff, I. A., *Lipids*, 1966, **1,** 335
7. Morris, L. J., & Hall, S. W., *Lipids*, 1966. **1,** 188
8. Gunstone, F. D., *J. chem. Soc.*, 1952, p. 1274; *J. Sci. Fd Agric.*, 1952, **3,** 185; 1953, **4,** 129; with Morris, L. J., 1959, **10,** 522
9. Stoffel, W., Chu, F., & Ahrens, E. H., *Analyt. Chem.*, 1959, **31,** 307
10. Wood, R. D., Raju, P. K., & Reiser, R., *J. Am. Oil Chem. Soc.*, 1965, **42,** 81, 161
11. Gunstone, F. D., & Padley, F. B., *J. Am. Oil Chem. Soc.*, 1965, **42,** 957

EVALUATION OF MAIZE GLUTEN MEAL—PERUVIAN FISH MEAL MIXTURES AS PROTEIN SUPPLEMENTS FOR EGG PRODUCTION

By N. JACKSON and E. SQUANCE

Maize gluten meal protein was used to replace 25, 50, 75 and 100% of the protein supplied by Peruvian fish meal (14·25%) in a cereal-based diet for caged hens.

When the level of maize gluten meal was increased to provide 50% of the supplementary protein, egg production dropped from 80% on the fish meal control diet to 75%, and fell to 53·4% when all the supplementary protein in the diet was provided by maize gluten meal. Mean egg weight dropped significantly when the dietary maize gluten meal supplied 75% and 100% of the supplementary protein. The best food conversion efficiencies were obtained on the two diets with the highest content of Peruvian fish meal. The energetic efficiency for egg production fell slightly when the contribution of dietary protein from the maize gluten was equal to that from the Peruvian fish meal, and fell markedly at the two highest levels of dietary maize gluten meal.

The conclusion is reached that satisfactory egg production can be maintained when up to 25% of the protein of the fish meal control diet is replaced by maize gluten meal protein, and that the efficiency of utilisation of energy for egg production is not adversely affected.

Introduction

The literature pertaining to the use of maize gluten meal as a protein supplement in the diet of laying hens has recently been reviewed.[1]

In a recent experiment using light hybrid pullets, the authors[1] have found that 15·25% maize gluten meal in the diet, supplemented with 0·20% L-lysine was capable of supporting about 80% of the egg production which was obtained using a similar diet in which white fish meal was the high protein source. In the United Kingdom, fish meal is one of the most widely used protein supplements and is a rich source of lysine, while maize gluten meal is low in lysine. Maize gluten meal has a consumer value in that it produces a deep egg yolk colour. In previous work[1] it was found that all levels of maize gluten meal in excess of 8% resulted in eggs which had the same mean yolk colour score.

In the experiment reported below Peruvian (anchovy) fish meal was used with maize gluten meal in varying proportions in order to assess the optimum level of maize gluten meal which can be used in layers' diets in which these two meals are complementary sources of amino acids.

Experimental

The experiment was started in September 1966 and continued for nine 28 day periods to June 1967. The first 28 day period was a pre-experimental period. During the 8 experimental periods individual records of egg weight, egg numbers and feed intake were kept for each hen. Initial and final body weights were also recorded.

Two hundred light hybrid pullets were housed in individual cages equipped with individual feed troughs and communal drinkers. At the time of transference of the hens to individual laying cages the hens were being subjected to 10 hours' light daily and this was increased by one hour per week over a period of 7 weeks to a final lighting period of 17 hours.

The pullets were randomised into five treatment groups of 40 birds per group, and each group was fed one of the five experimental diets. The diets fed were:

A. A high-energy diet containing 14% Peruvian fish meal as the main protein source (fish meal control).
B. The same diet with 25% of the Peruvian fish meal protein replaced by maize gluten meal protein (75% PFM, 25% MGM).
C. The same diet with 50% of the Peruvian fish meal protein replaced by maize gluten meal protein (50% PFM, 50% MGM).
D. The same diet with 75% of the Peruvian fish meal protein replaced by maize gluten meal protein (25% PFM, 75% MGM).
E. The same diet with 15% maize gluten meal and no fish meal.

All the diets were designed to contain the same total crude protein level and similar metabolisable energy (ME) levels and to have only very small differences in the cereal components.

The composition, determined ME, crude protein, calcium and phosphorus contents are given for each of the diets in Table I.

The amino acid compositions of the five diets are presented in Table II, together with the A.R.C.[2] recommended amino acid requirements for laying hens. The data for the amino acid composition of the maize gluten meal were supplied by the manufacturers[3] and those for the Peruvian (anchovy) fish meal by the Peruvian Government.[4] The data for all other constituents were taken from Bolton's[5] figures. The ME content of each diet for adult fowl, was determined for two mixes, and the crude protein, Ca and P values are the mean values of three mixes of the diets.

The relative efficiency of utilisation of ME for egg production was calculated using the known daily ME intake and the estimated requirement calculated using the equation $M = 1{\cdot}86\,E + 115{\cdot}1\,W$, where M = ME intake (kcal/day), W = body weight (kg), E = egg product (g/day) + change in body weight (g/day).

This equation was derived for White Leghorn hens by Waring (personal communication) from the calorimetric

work of Waring & Brown[6] and has been applied here as being the most suitable existing equation which could be applied to the light hybrid hens used in the present experiment. Energetic efficiency was calculated from the equation:

$$\text{Energetic efficiency} = \frac{\text{Gross energy in eggs} \times 100}{\text{ME of food}}$$

assuming the energy content of egg product to be 1·62 kcal/100 g.

Results

The mean results for egg production and feed conversion efficiency are presented in Table III together with the results of the observations on egg yolk colour.

The effect of diet on egg production was highly significant ($P < 0{\cdot}001$). The highest levels of egg production were obtained from the diets containing no maize gluten meal (Diet A) and the lowest level of maize gluten meal (Diet B). When the level of maize gluten meal was increased to provide 50% of the supplementary protein (Diet C) then egg production dropped from over 80% to 75%, an effect which is on the borderline of statistical significance but is considered to be a definite effect of dietary treatment. The difference in egg production between hens fed diets C and D (50% and 75% MGM respectively) was not significant, but production on Diet D was significantly lower than on diets A and B. When all the main protein-containing constituent of the diet was present as maize gluten meal (Diet E), egg production fell to 53·4%, a value which was markedly lower than that obtained from any of the other dietary treatments.

The results for total egg weight follow the same pattern as those obtained for egg number. There was no significant difference in the total egg weight obtained when the two diets lowest in maize gluten meal content (Diets A and B) were fed, but when the contribution from maize gluten meal protein was equal to that of the fish meal (Diet C), total egg weight fell significantly, and continued to fall with the increase of the percentage of maize gluten meal in the diet. Mean egg weight was not significantly different for the three diets lowest in maize gluten meal content, but when the level of maize gluten meal was increased to 75% of the total protein supplement, egg weight fell significantly; this effect was very marked on the diet containing no fish meal, which

TABLE I

Percentage compositions of the diets

	A Fish meal control	B 75% PFM 25% MGM	C 50% PFM 50% MGM	D 25% PFM 75% MGM	E Maize gluten meal control
Maize meal, 9·0% CP	55·50	55·50	55·25	55·25	55·00
Ground wheat, 10·0% CP	20·50	20·25	20·25	20·00	20·00
Peruvian fish meal, 64·5% CP	14·25	10·70	7·15	3·55	—
Maize gluten meal, 61·9% CP	—	3·80	7·60	11·45	15·25
Ground limestone	7·00	7·00	7·00	7·00	7·00
Steamed bone flour	2·00	2·00	2·00	2·00	2·00
Common salt	0·50	0·50	0·50	0·50	0·50
*Mineral-vitamin supplement	0·25	0·25	0·25	0·25	0·25
Total	100·00	100·00	100·00	100·00	100·00
Nitrogen-corrected ME, kcal/kg	2740	2730	2810	2840	2880
Standard ME, kcal/kg	2830	2820	2890	2900	2950
Crude protein, %	16·3	16·2	17·1	16·4	17·2
Calorie : protein ratio	174	175	169	177	171
Ca, %	4·82	4·57	4·23	3·63	3·52
P, %	1·10	0·82	0·75	0·63	0·52

* The mineral-vitamin supplement contained $3{\cdot}52 \times 10^6$ I.U. Vitamin A, $0{\cdot}88 \times 10^6$ I.U. Vitamin D_3, 1·76 g Vitamin B_2, 3·0 mg Vitamin B_{12}, 3·52 g Vitamin E, 0·44 g Vitamin K, 8·8 g nicotinic acid, 3·52 g pantothenic acid, 0·22 g folic acid, 44 g choline chloride, 8·81 g Fe, 1·32 g Co, 44·1 g Mn, 2·20 g I, 44·1 g Zn and 3·52 g Cu per kg.

TABLE II

Amino acid requirements and the calculated amino acid contents of the diets shown in Table I—(% of diet)

	A.R.C. Requirements	A Fish Meal Control	B 75% PFM 25% MGM	C 50% PFM 50% MGM	D 25% PFM 75% MGM	E Maize Gluten Meal Control
Arginine	—	0·98	0·93	0·89	0·84	0·79
Glycine	—	0·89	0·77	0·65	0·53	0·42
Histidine	—	0·45	0·43	0·42	0·40	0·38
Leucine	0·70	1·61	1·75	1·90	2·05	2·19
Iso-leucine	0·50	0·86	0·83	0·81	0·78	0·76
Lysine	0·50	1·06	0·90	0·75	0·59	0·43
Methionine + cystine	0·55	0·74	0·73	0·72	0·71	0·70
Phenylalanine + tyrosine	0·70	1·14+	1·26+	1·37+	1·50+	1·62+
Threonine	0·40	0·91	0·84	0·78	0·71	0·64
Tryptophan	0·13	0·22	0·20	0·18	0·16	0·14
Valine	0·55	1·21	1·15	1·09	1·02	0·96

gave a mean egg weight 5·6 g lower than that obtained from the fish meal control diet.

The best food conversion efficiencies were obtained from the fish meal control and the 25% maize gluten meal diet, and this figure deteriorated as the level of maize gluten meal in the diet increased. This effect was significant for the two diets with the highest level of maize gluten meal when they were compared with diets A or B.

The results for egg yolk colour show that all the diets resulted in eggs with an acceptable yolk colour, and the colour score rose with increasing maize gluten meal content in the diet. The relatively high score of the fish meal control diet is due to the high maize content of the basal ration.

Data for mean total and daily feed intake, mean daily ME intake, relative efficiency of utilisation of ME and mean daily crude protein intake are presented in Table IV together with the mean bodyweight data.

The feed intakes were not significantly different for any of the four diets containing fish meal, but fell significantly when all the fish meal was replaced by maize gluten meal. The relative efficiency of utilisation of ME for egg production was highest for the two diets highest in fish meal, and fell steadily, but not very markedly, with increasing levels of maize gluten meal in the diet. The energetic efficiency for egg production showed a fall when the contribution of protein from maize gluten meal was reduced until it was approximately equal to that from fish meal (Diet C) and it fell markedly when all the fish meal protein was replaced by maize gluten meal.

Discussion and Conclusions

The object of the experiment was to find what level of maize gluten meal would successfully replace a portion of the fish meal in layers' rations. The results indicate that up to 25% of the protein of the fish meal control diet can be replaced by maize gluten meal protein with no drop in egg production or in the efficiency of utilisation of energy.

The drop in egg production which occurred when the maize gluten protein was increased to 50% or more of the total high protein supplement may possibly be attributed to an imbalance of amino acids, although at this level none of the amino acids presented in Table II is deficient.

A marked effect of increasing the level of dietary maize gluten meal is the increase in the ratios of leucine to iso-leucine and leucine to valine. A similar effect can be seen in the ratio of arginine to lysine. Smith & Lewis[7] have described an agent–target interaction for lysine and arginine, and a similar interaction has also been reported for leucine, iso-leucine and valine.[8] Although their work has been based on growth experiments it is not unreasonable to assume that these interactions are valid for egg production also. At the highest level of maize gluten meal the data indicate a deficiency of lysine while the tryptophan figure is approaching a threshold value.

The mean daily ME intake values for all the fish meal diets were similar (mean 315 kcalME) but there was a marked fall to 268 kcal ME per day when all the fish meal was replaced by maize gluten meal. The ME content of this latter diet was similar to that for diets C and D and considerably higher

TABLE III

Mean egg production, food conversion efficiency and egg yolk colour data

	A FMC	B 75% PFM 25% MGM	C 50% PFM 50% MGM	D 25% PFM 75% MGM	E MGM	Approximate standard error of a mean
Mean eggs per bird	180	180	168	164	119	4·074
Mean percentage production	80·2	80·4	75·0	73·4	53·0	1·819
Mean total egg weight per bird, kg	9·814	9·981	9·178	8·650	5·795	0·215
Mean egg weight, g	54·7	55·4	54·6	52·9	49·1	0·535
Mean food conversion, kg feed/kg eggs	2·55	2·50	2·72	2·94	3·79	0·100
Mean egg yolk colour (Fletcher's Rings System)	9	11	12	12	13	—

TABLE IV

Mean body weight data, food, ME and crude protein intakes and energy conversion data

	A Fish Meal control	B 75% PFM 25% MGM	C 50% PFM 50% MGM	D 25% PFM 75% MGM	E Maize gluten meal control	Approximate standard error of a mean
Mean body weight, kg						
Initial	1·59	1·61	1·63	1·61	1·53	0·025
Final	1·87	1·88	1·89	1·86	1·55	0·034
Mean body weight gain, kg	0·28	0·27	0·26	0·24	0·01	0·024
Mean total food intake per bird, kg	24·86	24·74	24·36	24·80	20·49	0·341
Mean daily food intake, g	111	110	109	111	91	1·523
Mean daily crude protein intake, g	18·1	17·8	18·7	18·2	15·7	—
Mean daily ME intake, kcal	314	310	315	322	268	—
Mcal of ME/kg eggs	7·17	6·99	7·67	8·31	10·43	—
Relative efficiency of utilisation of ME for egg production	90	92	89	85	84	—
Energetic efficiency	22·6	23·2	21·1	19·5	15·5	—

than those of diets A and B, and it would appear from this that the effect is due to the imbalance and deficiency of amino acids in the diet. This effect has been noted in a previous experiment.

The effect of diet on egg size may also be attributed to amino-acid level in the diet. Such an effect has been shown by March & Biely.[9] The adverse effect on egg production and egg size found with diet E may also be attributed partially to the lowered ME intake.

Brown, Waring & Squance[10] concluded that a C : P ratio of 167–172 (kcal ME/kg/% protein) gave a maximum efficiency of food conversion. In the present experiment the C : P ratios of the diets are in the range 169–172. It should be noted that in the earlier publication[10] the ratio was derived using nitrogen-corrected ME values, but in the present paper standard ME has been used. The ratios for diets of normal crude protein content (16–19%), when calculated using standard ME values, are 4–5 units higher than when calculated using the nitrogen-corrected values. Therefore, in the present experiment the C : P ratios of the diets are similar to those recommended,[10] and it is unlikely that the small variations in C : P ratio have affected the results of the present experiment.

Although the Ca and P contents of the diets decrease considerably with increasing level of maize gluten meal, it is considered that the contents of these minerals were adequate in all the diets.

The small improvement in production and overall efficiency by the hens fed 25% maize gluten meal protein was not statistically significant, but may nevertheless be real and have an economic significance.

With regard to egg yolk colour, maize gluten meal would probably be of more value in diets high in milo, wheat or barley, than in diets high in maize, which has a high xanthophyll content.

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Department of Agricultural Chemistry,
Queen's University,
Belfast BT9 6BB, and
Ministry of Agriculture
Northern Ireland

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References

1. Squance, E., & Jackson, N., *J. Sci. Fd Agric.*, 1967, **18,** 405
2. 'The nutrient requirements of farm livestock, 1. Poultry', 1963. (London: Agricultural Research Council)
3. Corn Products (Sales) Ltd., Broadsheet Number FE/1/867
4. 'Peruvian Fishmeal'. (Consorcio Pesquero del Peru: Lima, Peru)
5. Bolton, W., *Poult. Nutr. Bull.* 174, 1964, 2nd edn., with amendments (London: HMSO)
6. Waring, J. J., & Brown, W. O., *J. agric. Sci.*, 1967, **68,** 149
7. Smith, G. H., & Lewis, D., *Br. J. Nutr.*, 1966, **20,** 621
8. Spolter, P. D., & Harper, A. E., *Am. J. Physiol.*, 1961, **200,** 513
9. March, B. E., & Biely, J., *Nature, Lond.*, 1963, **200,** 702
10. Brown, W. O., Waring, J. J., & Squance, E., *Br. Poult. Sci.*, 1965, **6,** 59

NOTE ON VARIATIONS IN THE MINERAL COMPOSITION OF OAT AND BARLEY GRAIN GROWN IN WALES

By D. E. MORGAN

Analytical results for the major elements, Ca, Mg, Na, K, P and Cl, and the trace elements, Cu, Co, Mn and Zn, for approximately 170 samples each of oat and barley grain from 3 seasons, are presented. The variations in contents are discussed in relation to current dietary recommendations for farm livestock.

Introduction

In a previous paper[1] attention was drawn to variations in the proximate constituents and estimated energy values of oat and barley grain grown in Wales. The present report is concerned with variations in the contents of major and trace elements of importance in livestock nutrition.

Experimental

Details of the survey have been given in the earlier paper.[1] Samples of the same oat and barley grain were analysed for the elements listed below. The analytical methods which are outlined below, are those in normal routine use in this laboratory and full details of them can be obtained from the writer.

Ca and Mg —titrimetrically using EDTA.
Na and K —by flame photometry in hydrochloric acid solution.
P — colorimetrically as ammonium phosphomolybdate.
Cl — titrimetrically following precipitation as silver chloride and back titration of excess silver nitrate with potassium thiocyanate.
Cu — colorimetrically as copper dibenzyldithiocarbamate.
Mn — colorimetrically as permanganate following periodate oxidation.
Zn — colorimetrically as zinc dithizonate.
Co — colorimetrically as cobalt-2-nitroso-1-naphthol.

Results

Major elements

The summarised results for the contents of major elements in all grain samples analysed over a three year period are given in Table I.

The ranges of values recorded indicate that considerable variation exists in the contents of all major elements in both grains. This is further illustrated by the high coefficients of variation, which ranged from 8·3% for Mg in barley to 47·8% for the Na content of oats. Detailed comparisons between years did not indicate any seasonal differences in either oats or barley. Oats were found to have a slightly but significantly higher Ca content than barley while the latter was significantly higher in Cl. No other differences were observed. It may be noted that both cereals contain higher amounts of Mg than of Ca, while both are very low in Na.

Further statistical analysis did not reveal any significant correlations between the various major elements.

Trace elements

The contents of the four trace elements, Cu, Co, Mn and Zn which are currently considered as of considerable practical importance in farm animal nutrition are summarised in Table II.

Again it will be noted that considerable variation was found in the contents of all elements, especially Co, as indicated by the ranges of values and high coefficients of variation.

TABLE I

Major-element contents of Welsh oat and barley grain, 1961–63
(as % in dry matter)

	Oats 171 samples			Barley 179 samples		
	Mean	Range	% Coefficient of variation	Mean	Range	% Coefficient of variation
Ca	0·11	0·07 –0·18	18·2	0·08	0·05 –0·16	25·0
Mg	0·13	0·10 –0·18	13·1	0·12	0·09 –0·16	8·3
K	0·47	0·31 –0·65	17·0	0·49	0·35 –0·63	12·2
Na	0·02	0·004–0·06	47·8	0·02	0·006–0·04	41·2
P	0·38	0·29 –0·59	10·5	0·38	0·26 –0·52	11·8
Cl	0·09	0·04 –0·18	33·3	0·14	0·08 –0·22	21·4

TABLE II

Trace-element content of Welsh oat and barley grain, 1961–63
(as ppm in dry matter)

	Oats				Barley			
	Number of samples	Mean	Range	% Coefficient of variation	Number of samples	Mean	Range	% Coefficient of variation
Cu	171	4·7	3·0 –8·2	17·0	179	6·6	3·5 –19·8	27·3
Co	163	0·05	0·02–0·17	53·0	174	0·07	0·02–0·18	48·6
Mn	171	45	22–79	28·9	179	16	5–47	31·3
Zn	171	37	21–70	27·0	179	37	19–77	27·0

Oats and barley would supply similar total amounts of Cu, Co and Zn, but oats, on average contains nearly three times as much Mn as barley. Again no seasonal differences were observed in the trace element contents of either oats or barley, neither were there any significant correlations between individual trace elements or between them and any of the major elements such as Ca.

Examination of all the data for both major and trace elements did not reveal any varietal differences, neither was it possible to relate variations in the contents of any of the constituents to soil type or fertiliser practice.

Discussion

The purpose of this study was to provide information on variations existing in the composition of oat and barley grain as grown and fed on Welsh farms, and to comment on their possible nutritional implications.

As far as the major nutrients are concerned the recorded mean values are of a similar order to those given in Ministry of Agriculture Bulletins No. 48 'Rations for livestock'[2] and No. 174 'Poultry nutrition'[3], although the Welsh barley samples appear to be slightly higher in Ca. The present results confirm the well known fact that these cereals are poor sources of major nutrients and when fed to all classes of stock require supplementation with sodium chloride and Ca and P compounds.

A knowledge of variations in the K contents may have practical significance in view of the conclusion in A.R.C. Technical Report No. 2 (Ruminants)[4] that K deficiency may occur in older store cattle fed diets consisting almost entirely of cereals. Such a situation would arise in intensive 'barley beef' feed lots. The current A.R.C. estimates of the K and Mg requirements of pigs,[5] and chicks,[6] indicate that both cereals contain adequate amounts of these elements.

In the case of Mg, both grains would in general supply adequate amounts for growing and fattening cattle and sheep, but when fed in the usual amounts, would not provide more than about one third of the A.R.C. estimated requirements for lactating cattle and sheep. Thus a 500 kg Friesian cow yielding 20 kg of milk is estimated to require 20 g Mg per day. In addition to forage and protein supplements such an animal might typically receive 12 lb of cereals, which would provide on average only about 6 g of Mg per day. The balance could easily be provided by good forage but with modern diets of barley straw and concentrates, additional Mg supplementation is probably desirable.

The present results show that both grains have similar Co contents, and barley is marginally a better source of Cu than oats, but both cereals are low in these elements, particularly when related to the A.R.C.[4] estimated requirements of 10 ppm and 5 ppm of Cu for cattle and sheep, and 0·1 ppm of Co for both species. Moderate supplementation of cereals with Cu would seem to be desirable particularly in view of the generally low content in average quality roughages and the fact that it is not uncommon to record cereal Cu values below the low-level requirements suggested for sheep. Co supplementation is also desirable because only 19% of the barley samples and 10% of the oats contained 0·1 ppm or more Co.

So far as growing pigs are concerned, both cereals contain Cu levels in excess of the 4 ppm proposed by the A.R.C.[5] However, this is mainly of academic interest in view of the use of copper sulphate as a growth stimulant in fattening pig diets. The Cu requirement of 3·2 mg/kg of diet[6] advised for chicks is also easily met by both grains.

The Mn and Zn contents of oats found here are similar to those reported by Dewar,[7] in his review of the Zn and Mn contents of some British poultry feeds, but although his mean value of 18 ppm for Mn in barley is close to the mean found for Welsh samples, his value of 27 ppm for zinc is below that found in this study. It should be noted however that his results are for 4 samples only.

For ruminants the A.R.C.[4] recommend a Mn dietary level of 40 mg/kg dry matter. Barley grain therefore is a poor source of Mn, and supplementation for lactating ruminants is desirable, especially when diets containing limited low-grade roughage and large quantities of barley are being fed. Neither cereal is a good source of Mn for poultry, but barley is particularly poor, especially in view of the commonly accepted practical recommendations of Bolton[3] that feeds should contain 55 mg/kg dry matter for chicks and 70 mg/kg dry matter for laying and breeding fowls. These suggestions are rather higher than the A.R.C.[6] value of 35 ppm Mn. From the limited information available it seems that both cereals are reasonable sources of Mn for pigs.[5]

The mean Zn contents found for Welsh oat and barley grain are well below the A.R.C.[4,5] recommended levels of 50 mg/kg dry matter for the diets of cattle, sheep and pigs, and despite considerable variations in content these grains must be suspect as adequate Zn sources. The picture with poultry is again confused because Bolton[3] and the A.R.C.[4] recommend different levels. The A.R.C. report points out the difficulties of assessing Zn requirement since soyabean protein and high Ca in diets may render Zn unavailable. The higher levels suggested by Bolton therefore appear to be justified and it seems that a high proportion of Welsh oats and barley would contain less Zn than required.

Finally, it may be noted that the variable and frequently low content of Zn in barley offers a partial explanation for the occurrence of Cu toxicosis in simplified pig diets, which may consist of 85–90% of barley. Hanrahan & O'Grady[8] have reported a high incidence of Cu toxicosis when such diets are supplemented with 0·1% copper sulphate to act as a growth stimulant, but the effect can be counteracted by the addition of 125 ppm Zn as zinc carbonate. Similar effects have been noted by Suttle & Mills[9] using diets in which maize is the principal cereal. They also found that added Fe assists in the prevention of copper toxicosis. Despite the addition of copper sulphate, problems of this type do not seem to be prevalent in commercial practice when traditional meal mixtures for pigs containing 25–30% of wheat feed are used. Replacement of wheat feed by barley grain substantially reduces the Zn content of the diet because, as Dewar[7] has shown, the former is a good source of Zn. Suttle & Mills[9] have already suggested that variations in the content and availability of dietary Zn and Fe may have contributed to isolated cases of Cu toxicity in pigs fed copper sulphate supplemented diets.

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Nutrition Chemistry Department,
N.A.A.S. Sub-Centre,
Ministry of Agriculture, Fisheries and Food,
Bryn Adda,
Penrhos Road,
Bangor, Wales

Received 1st January, 1968

References

1. Morgan, D. E., *J. Sci. Fd Agric.*, 1967, **18**, 21
2. *Bull. Minist. Agric. Fish Fd. No. 48*, 1961
3. Bolton, W., in *Bull. Minist. Agric. Fish Fd No. 174*, 1963
4. Agricultural Research Council 'The nutrient requirements of farm livestock No. 2. Ruminants', 1965 (London: A.R.C.)
5. Agricultural Research Council 'The nutrient requirements of farm livestock No. 3. Pigs', 1967 (London: A.R.C.)
6. Agricultural Research Council 'The nutrient requirements of farm livestock No. 1. Poultry', 1963 (London: A.R.C.)
7. Dewar, W. A., *J. Sci. Fd Agric.*, 1967, **18**, 68
8. Hanrahan, J. F., & O'Grady, J. F., *Ann. Rep. Anim. Prod. Div. An Foras Taluntais*, 1965 & 1966
9. Suttle, N. F., & Mills, C. F., *Br. J. Nutr.*, 1966, **20**, 135

EFFECTS OF GAMMA RADIATION ON ACCUMULATION OF MINERAL NITROGEN IN FRESH SOILS

By P. A. CAWSE

Increases in mineral nitrogen in gamma-irradiated soils have been investigated in relation to dose over the range 0·05–3 Mrad, especially with regard to formation of nitrate. All soils showed a significant increase in nitrate-N after irradiation, but the response was much greater in the most organic series and reached a maximum after 0·4 Mrad; it was followed by a rise in nitrite at 0·6 Mrad. The amount of radiation that gave maximum accumulation of nitrate-N varied between soils, probably owing to differences in the surviving population of nitrifying bacteria.

Perfusion of soil with ammonium ions showed that rapid nitrification after doses greater than 0·1 Mrad was most likely to result from oxidation by non-proliferating cells of the nitrifying bacteria rather than increased proliferation of the survivors.

Introduction

It has been reported[1] that increases in ammonium- and nitrate-N are a sensitive index of response of soils to radiation, and the extra mineral N produced can give rise to increases in yield when plants are grown in irradiated soil.[2–4]

When five fresh soils were treated with 0·02 and 0·2 Mrad of gamma radiation followed by one week of incubation, the nitrification process was greatly stimulated in all of them; nitrate production after 0·2 Mrad increased as much as ten-fold compared with the control,[1] and the present investigation was undertaken with two main objects: firstly, to determine the radiation dose that would give rise to maximum stimulation of nitrification in the soil types used for the previous study, above which an inhibition of ammonium

oxidation would occur. Secondly, the soil perfusion technique[5] was used to establish whether increased proliferation of the nitrifying bacteria that survived irradiation could be responsible for the extra nitrate production, rather than increased oxidation of ammonium substrate by non-proliferating cells.

Experimental

Materials

The soils were sampled from the top 6 in of the Black, Broad, Faringdon, Grove and Hanney series, all under arable cultivation; their properties have already been described.[1] They were passed through a 2 mm sieve, and distilled water was added to obtain a moisture level between 60–70% of field capacity. After equilibration for two days at room temperature, 70 g samples were irradiated with a source of ^{60}Co giving a dose rate of 0·7 Mrad/h; soil containers remained unopened until the end of incubation.

Initially, Grove soil was exposed to a range of doses between 0·05–3·0 Mrad; ammonium-, nitrite- and nitrate-N were determined before irradiation, and after 7 days of incubation at 25°. Analysis was also made after 14 days with the samples treated over 1–3 Mrad. The results (Fig. 1) indicated that nitrification was inhibited by doses greater than 0·6 Mrad; consequently the other series were only treated up to 1 Mrad and incubated for 7 days, in the hope that they would show maximum nitrification response in a similar dose region.

For perfusion experiments, the Audus modified apparatus[6] was selected. Broad and Grove soils were air-dried at 25°, and 20 g of the 0·6–2 mm fraction were placed in 3·5 × 10 cm. nylon (250 micron mesh) sachets for irradiation; the full sachet was a close fit into the vertical tube of the perfusion unit. This technique avoided excessive handling of soil after irradiation, and also radiation discoloration of the units which would prevent observation of conditions in the perfusate.

Samples were treated with 0·0125, 0·025, 0·05, 0·1 and 0·2 Mrad, and immediately placed in heat-sterilised perfusion units each containing 250 ml 0·01M ammonium sulphate which had been passed through a bacteriological membrane filter with a pore size of 0·5 μm. Units were placed in an incubator at 21°, and connected between two manifolds; one led to an air suction pump and the other, on the incoming side, was connected to two gas washing bottles, each containing 250 ml water, to clean and humidify the air.

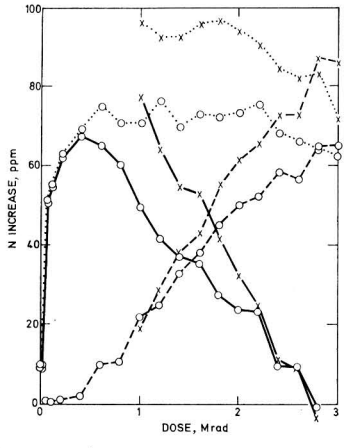

FIG. 1. *Increases in the concentrations of ammonium* (— —) *nitrate* (——) *and total mineral-N* (. . . .) *in Grove soil after* 7 (○) *and* 14 (×) *days of incubation at* 25°C

Soils were not perfused to obtain bacterial saturation[7] with nitrifying organisms before irradiation, because it was desirable to start with population numbers as near as possible to incubation tests and proliferation would be clearly seen when perfusion started. During perfusion, the perfusates were kept at pH 7·5 by addition of N-KOH; treatments that did not show recovery of nitrification by proliferation up to 20 days were perfused for a further period.

Method of analysis

Exchangeable ammonium- and nitrate-N in incubated samples were extracted by shaking 50 g fresh soil for one hour with 100 ml acidified N-KCl and 0·25 ml toluene; the filtrate was analysed by the distillation method of Piper.[8]

Nitrate-N in 0·1–0·2 ml aliquots of perfusates was determined by the rapid ultra-violet spectrophotometric method of Cawse.[9]

Nitrite-N in soils was determined by a modified Griess–Ilosvay technique;[10] 10 g soil was shaken for 30 minutes with 100 ml water at neutral pH, and the filtrate was analysed.

Results

The mineral-N response of Grove soil to a wide range of radiation doses is presented in Fig. 1. Nitrification showed maximum stimulation in the 0·4 Mrad region, and was only reduced to the control rate (9·1 ppm nitrate-N in 7 days) by 2·5 Mrad. It is known that 70 ppm of ammonium-N in Grove soil is rapidly nitrified,[1] therefore the decline in nitrification above 0·4 Mrad cannot be attributed to the toxicity of radiation-released ammonia.

The mineral-N response of other irradiated soil series is shown in Table I. All gave significant increases in nitrate-N over control during incubation, but the Broad series (6·6% total C) produced a larger response than Black (3·5% total C), Faringdon (2·2% total C), Grove (3·5% total C) or Hanney (3·2% total C) soils. The peak of maximum nitrification for each series did not always occur at the same radiation dose, and an unusual feature, which has been discussed in a preliminary note,[11] was the accumulation of nitrite in Broad soil after 0·6–1·0 Mrad.

The results from perfusion of Broad and Grove soils are presented in Figs 2 and 3 respectively. Proliferation was evident in the controls by rapid initial production of nitrate (logarithmic phase), but a linear rate corresponding to a state of bacterial saturation in the soil was soon achieved;[7] this continued as long as substrate was available. The logarithmic increase in biochemical activity suggests that nitrification was carried out by chemoautotrophic bacteria rather than heterotrophic organisms.[12] Treatment with 0·1 and 0·2 Mrad prevented proliferation of nitrifying bacteria for the first 15 days of perfusion in both soils, but there was some oxidation of ammonium. The rate of oxidation in irradiated soils before proliferation had started was either constant or decreasing slowly: it appears that the irradiated population can still oxidise ammonium, although unable to proliferate in the normal manner. Consideration of these

TABLE I

Increase in ammonium-, nitrite- and nitrate-N (ppm N) over control in irradiated soils during 7 days' incubation at 25°C

Megarads	Soil series and form of mineral-N								
	Black†		Broad			Faringdon†		Hanney†	
	NH_4-N	NO_3-N	NH_4-N	NO_2-N	NO_3-N	NH_4-N	NO_3-N	NH_4-N	NO_3-N
0·05	1·9	38·2**	0·2	0·2	57·1***	0·5	18·2**	6·4*	19·6*
0·1	3·1	47·5**	0·2	0·4	87·0***	0·5	25·7***	9·8**	17·2*
0·2	3·0	50·0***	1·2	0·4	112·8***	2·6	26·2**	17·3**	17·8*
0·4	3·7	54·7***	0·3	0·7	128·2***	4·3	27·2**	24·7**	17·2*
0·6	3·8	60·1***	1·4	27·5***	95·8***	12·8**	23·6**	27·0**	15·1*
0·8	3·7	66·9***	3·1	34·7***	77·8***	16·0**	21·4**	28·3**	11·6
1·0	6·2*	65·1***	5·6	32·9***	66·7***	20·8**	18·8**	29·9**	9·2
Control increase	(−0·4)	12·7	2·6	0·5	13·4	1·6	3·5	(−0·8)	9·6

†NO_2-N omitted in three soils where increase was <0·3 ppm after all doses: control increase was <1 ppm NO_2-N
Significantly different from control at * 5% level
** 1% level
*** 0·1% level

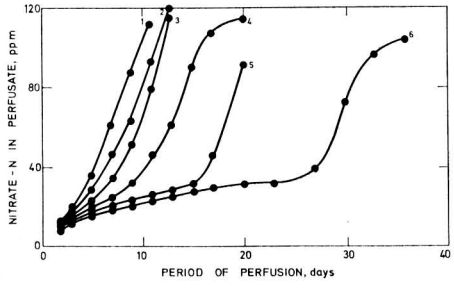

FIG. 2. *Post-irradiation recovery of nitrification in Broad soil, perfused with ammonium*

1 control
2 0·0125 Mrad
3 0·025 Mrad
4 0·05 Mrad
5 0·1 Mrad
6 0·2 Mrad

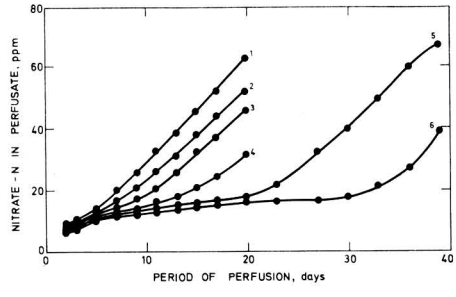

FIG. 3. *Post-irradiation recovery of nitrification in Grove soil, perfused with ammonium*
Key as for Fig. 2.

findings in relation to Fig. 1 (Grove series) and Table I (Broad series) reveals that 0·1 Mrad would prevent proliferation well below the dose that had been required for maximum nitrification in soil incubation tests over 7 days. Sufficient agreement was obtained between results from incubation and perfusion experiments (when the latter was approximately calculated on the basis of soil air-dry weight, i.e. $\frac{\text{ppm nitrate-N in perfusate} \times 250\ (\text{ml})}{20\ (\text{g})}$) to permit this conclusion. For example, after 0·2 Mrad and 7 days of incubation, the absolute nitrate-N increases for comparison of perfusion and incubation were 140 and 126 ppm, respectively, for Broad soil, and 54 and 62 ppm, respectively, for the Grove type.

Compared with the control soil, radiation always decreased nitrification in perfusion work but had the opposite effect with soil incubation, unless very large doses were given. In perfusion experiments, nitrification was not limited by lack of ammonium, in contrast to incubation experiments where the additional ammonium released by irradiation resulted in stimulation of nitrification.

There was recovery of nitrification at the higher doses when perfusion was continued up to 39 days. Figs 2 and 3 show that recovery occurred in the expected order in relation to dose, which is consistent with eventual proliferation of survivors rather than accidental contamination by nitrifying organisms.

In a perfusion test, on addition of fresh Grove soil to some that had received 0·2 Mrad, there was considerable recovery of nitrification after 11 days of perfusion, and the rate of ammonium oxidation reached 84% of that in the unirradiated control; inhibition from toxic compounds can therefore be dismissed as a major cause of decreased nitrification in relation to control in perfusion work, and direct radiation damage to the organisms appears to be the most important factor.

Discussion

It has been stated[1] that a distinction must be made between radiosensitive bacterial proliferation and more radioresistant cell oxidation processes, and the present study demonstrates

that the latter are responsible for significant changes in mineral-N following irradiation.

The accumulation of nitrate in a soil which has been irradiated and incubated will depend on the amount of ammonium released, the number of survivors capable of utilising it as substrate, and perhaps the efficiency of the nitrifying species present.

Soil from the Broad series is the most organic, and may support a greater general microflora, as well as larger numbers of nitrifying bacteria than the other series. Such a soil would not only release much ammonium on irradiation, but it is also likely that more nitrifying bacteria would survive radiation treatment than in a soil with a smaller initial population, because sterilisation is an exponential process.[13]

Several experimental observations agree with the previous suggestion. Firstly, the control Broad soil showed more nitrification during incubation than did other series (Table I). Secondly, it allowed a faster maximum rate of nitrification than Grove soil at bacterial saturation (Figs 2 and 3). Thirdly, recovery of nitrification was faster in Broad soil than in Grove soil (Figs 2 and 3), and after 0·2 Mrad the ammonium oxidation by non-proliferating cells was greater in the Broad series; both factors are in accordance with the idea of a high initial population of nitrifying bacteria, resulting in more survivors after irradiation.

Variation in the radiation dose that gave maximum nitrate-N is best seen by comparison of Hanney and Black soils (Table I). If there was maximum nitrification in Hanney series (pH 5·5) after 0·05 Mrad, owing to ample substrate but few surviving bacteria, extra radiation would be expected to be inhibitory. Fewer survivors could result from a smaller natural population compared with Black soil (pH 7·0), as well as differences in radiosensitivity either between the same nitrifying bacteria in varied soil environments, or between separate bacterial species.

Nitrate still accumulated when Grove soil was incubated from 7 to 14 days after irradiation (Fig. 1), in agreement with a prolonged nitrate rise during experiments on plant growth in irradiated soil;[3] no doubt the other series would give a similar response.

The total mineral-N curve (Fig. 1) adds further evidence to reports of negligible damage to humus, compared with the biomass, from irradiation up to 2·5 Mrad;[14,15] death, lysis and ammonification of radiosensitive organisms in Grove soil are virtually complete at 0·6 Mrad.

At first, it was thought that greater radiation damage to organisms oxidising nitrite compared with those involved in oxidation of ammonium might lead to accumulation of nitrite, but subsequent work[11] revealed that nitrite in irradiated Broad soil (Table I) originates from nitrate reduction, and another organic calcareous soil from the Icknield series gave a similar response. This mechanism is of considerable interest in connexion with nitrogen losses from soil, [16,17] and is being further investigated.

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Wantage Research Laboratory,
U.K. Atomic Energy Authority,
Wantage, Berks.

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References

1. Cawse, P. A., *J. Sci. Fd Agric.*, 1967, **18,** 388
2. Bowen, H. J. M., & Cawse, P. A., 'Radio-isotopes in soil-plant nutrition studies', 1962, p. 17 (Vienna: International Atomic Energy Agency)
3. Bowen, H. J. M., & Cawse, P. A., *Soil Sci.*, 1964, **97,** 252
4. Cummins, D. G., & McCreery, R. A., *Proc. Soil Sci. Soc. Am.*, 1964, **28,** 390
5. Lees, H., & Quastel, J. H., *Biochem. J.*, 1946, **40,** 803
6. Sims, C. M., & Collins, F. M., *Analyst, Lond.*, 1958, **83,** 699
7. Lees, H., & Quastel, J. H., *Biochem. J.*, 1946, **40,** 815
8. Piper, C. S., 'Soil and plant analysis', 2nd edn., 1950, p. 206 and p. 92 (Adelaide: University Press)
9. Cawse, P. A., *Analyst, Lond.*, 1967, **92,** 311
10. Chapman, H. D., & Pratt, P. F., 'Methods of analysis for soils, plants and waters', 1961, p. 158 (California: University Press)
11. Cawse, P. A., & Crawford, D. V., *Nature, Lond.*, 1967, **216,** 1142
12. Alexander, M., 'Introduction to soil microbiology', 1961, p. 277 (New York: John Wiley & Sons Inc.)
13. Lea, D. E., 'Actions of radiations on living cells', 2nd edn., 1947, p. 72 (Cambridge: University Press)
14. Groenwood, H. van,. *Proc. Can. phytopath. Soc.*, 1958, No. 26, 12
15. Jenkinson, D. S., *J. Soil Sci.*, 1966, **17,** 280
16. Cooper, G. S., & Smith, R. L., *Proc. Soil Sci. Soc. Amer.*, 1963, **27,** 659
17. Allison, F. E., *Soil Sci.*, 1963, **96,** 404

ORGANOPHOSPHORUS COMPOUNDS

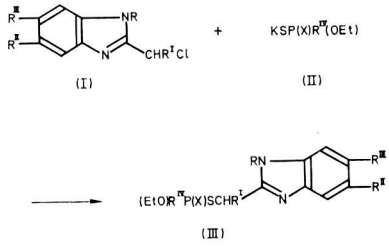
IV.*—2-[Diethoxy or ethoxy (ethyl) phosphinylthio- and diethoxy or ethoxy (ethyl) phosphinothioylthio-methyl] benzimidazoles

By M. PIANKA and J. D. EDWARDS

Several 2-chloromethylbenzimidazoles were prepared and condensed with potassium diethyl phosphoro- and phosphono- thiolothionates and thiolates for insecticidal and acaricidal studies.

Introduction

Because of the tautomeric nature of the imidazole ring[1] single phosphates (**III**) would be expected from condensations of the potassium salt (**II**) and 2-α-chloroalkylbenzimidazoles (**I**) with no substituents in the benzene ring or with substituents in the benzene ring only.



(I) (II) (III)

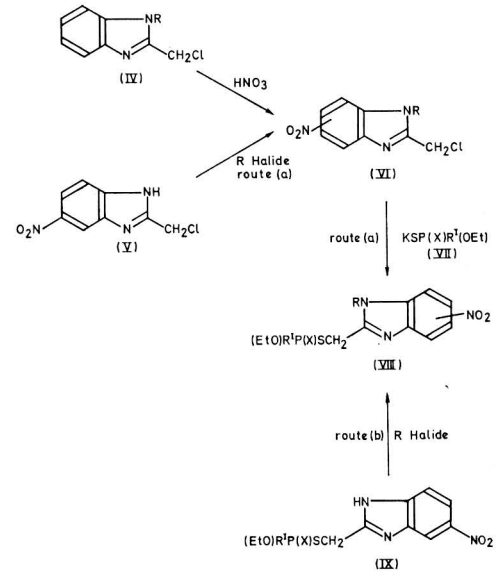
Mixtures of isomers (**VIII**) may, however, result when 1-substituted 2-chloromethylbenzimidazole (**IV**) is nitrated and then condensed with the potassium salt, or when 2-chloromethyl-5(6)-nitrobenzimidazole (**V**) is substituted with an organic radical on the nitrogen of the heterocycle and the resulting product (**VI**) is condensed with the potassium salt, or when the ring-substituted phosphate (**IX**) is substituted with an organic radical on the nitrogen of the heterocycle.

Experimental

Preparation of 2-chloroalkylbenzimidazoles (I)

2-Chloromethylbenzimidazole (*Compound 1*) (R, R^{I}, R^{II} and R^{III} = H)

2-Hydroxymethylbenzimidazole[2] (14·1 g) and thionyl chloride (55 ml) were heated until evolution of gases ceased (40 min). The volatile components were removed under reduced pressure and the residue was triturated with ether. The hydrochloride[3] (m.p. 248°, decomp.) was filtered off (17 g; 88%) and the base[4] was liberated from the hydrochloride on treatment with alkali.



(IV) (V) (VI) (VII) (VIII) (IX)

5(6)-Chloro-2-chloromethylbenzimidazole (*Compound 2*) (R, R^{I}, R^{III} = H; R^{II} = Cl)

This was prepared according to the method of Lettré *et al.*[3] The compound was obtained as brown crystals, m.p. 143° (from ether) (Found: N, 12·7. Calc. for $C_8H_6Cl_2N_2$: N, 13·0%).

2-Chloromethyl-1-methyl-5-nitrobenzimidazole (*Compound 3*) (R = Me; R^{I}, R^{III} = H; R^{II} = NO_2)

2-Hydroxymethyl-1-methyl-5-nitrobenzimidazole[5] (9·75 g) and thionyl chloride (50 ml) were heated under reflux for 30 min. Benzene (50 ml) was then added and the precipitated solid was filtered off, washed with benzene, dissolved in hot ethanol and treated with sodium bicarbonate until effervescence ceased. The mixture was then cooled, and the solid was filtered off and washed well with water. The

* Part III: Pianka, M., *J. Sci. Fd Agric.*, 1967, **18**, 63

compound (5·2 g; 46%) was obtained as white crystals, m.p. 196–197° (from acetone) (Found: Cl, 15·6. Calc. for $C_9H_8ClN_3O_2$: Cl, 15·7%). Ozegowski *et al.*[6] reported 191–192°.

2-(1-*Chloroethyl*)-5(6)-*nitrobenzimidazole* (*Compound 4*) (R, R^{III} = H; R^{I} = Me; R^{II} = NO_2)

This compound was prepared according to the method of Siegart & Day.[7]

2-*Chloromethyl*-5(6)-*nitrobenzimidazole* (*Compound 5*) (R, R^{I}, R^{III} = H; R^{II} = NO_2)

2-Chloromethylbenzimidazole hydrochloride (0·04 mole) was dissolved in conc. sulphuric acid (16 ml). A mixture of conc. sulphuric acid (4·8 ml) and conc. nitric acid (3·2 ml) was added dropwise, the reactants being cooled. The mixture was set aside for 3 h, then poured on to crushed ice and neutralised with sodium bicarbonate. The compound (V) (89%) had m.p. 186° (from 50% aqueous ethanol). Siegart & Day[7] reported m.p. 184–185°.

5(6)-*Chloro*-2-*chloromethyl*-6(5)-*nitrobenzimidazole*(*Compound 6*) (R, R^{I} = H; R^{II} = Cl; R^{III} = NO_2)

This was prepared by the nitration of Compound 2 according to the above method. The crude solid was crystallised from dilute hydrochloric acid and treated with aqueous sodium bicarbonate. The compound (65%) was obtained as yellow crystals, m.p. 210° (from toluene, then ethyl acetate) (Found: C, 38·8; H, 1·9; Cl, 28·9; N, 17·3. $C_8H_5Cl_2N_3O_2$ requires C, 39·0; H, 2·0; Cl, 28·9; N, 17·1%).

2-Chloromethyl-1-(alkoxy- or alkylthiolo-carbonyl) nitrobenzimidazoles (VI)

2-*Chloromethyl*-1-(*ethoxycarbonyl*)*nitrobenzimidazole* (*Compound 7*) (R = CO_2 Et)

A mixture of compounds, for which the position of the nitro-group was not determined, was prepared by either of two methods. The first was nitration of 2-chloromethyl-1-(ethoxycarbonyl)benzimidazole (**IV**; R = CO_2Et).[8] The oil that separated when the nitration mixture was poured on to crushed ice solidified into small and large crystals on standing. The mixture of crystals was recrystallised from ethanol, then from methanol to yield a yellow solid (78%) melting at 85–95° (Found: Cl, 12·5; N, 4·9†. $C_{11}H_{10}ClN_3O_4$ requires Cl, 12·5; N, 4·9%).

The other method was condensation of 2-chloromethyl-5(6)-nitrobenzimidazole with ethyl chloroformate. Pyridine (0·02 mole) was added dropwise to a refluxing solution of Compound 5 (V) (0·02 mole) and ethyl chloroformate (0·02 mole) in acetone (50 ml). The mixture was then heated under reflux for 30 min, treated with charcoal and kept at room temperature for 3 days. The mixture was then filtered. The acetone was distilled off from the filtrate, water and ether were added to the residue, and the mixture was shaken. The ether layer was separated, washed well with water, dried with anhydrous sodium sulphate, the ether was removed, and the residue was crystallised from ethanol then methanols to yield a solid melting at 89–98° (Found: C, 46·7; H, 3·4; Cl, 12·3; N, 15·4. $C_{11}H_{10}ClN_3O_4$ requires C, 46·6; H, 3·5; Cl, 12·5; N, 14·8%). On repeated crystallisation from 95% aqueous ethanol yellow-brown needles, m.p. 115–117°, and rosettes of needles, m.p. 105–106°, were obtained. The mixed m.p. of these needles was 89–98°; they may therefore be different isomers.

2-*Chloromethyl*-1-(*isopropoxycarbonyl*)*nitrobenzimidazole* (*Compound 8*) (R = CO_2 Pr^{i})

Compound 5 was condensed in a similar manner with isopropyl chloroformate. The residue which remained on evaporation of the ether yielded off-white prisms, m.p. 76–85° (from propan-2-ol, petroleum*, then methanol) (Found: N, 5·3†. $C_{12}H_{12}ClN_3O_4$ requires N, 4·7%).

2-*Chloromethyl*-1-(*butoxycarbonyl*) *nitrobenzimidazole* (*Compound 9*) (R = CO_2Bu)

Compound 5 was condensed with butyl chloroformate. The residue left on removal of the ether yielded yellow prisms, m.p. 58–65° (from methanol) (Found: N, 5·3†. $C_{13}H_{14}ClN_3O_4$ requires N, 4·5%).

2-*Chloromethyl*-1-(*methylthiolocarbonyl*) *nitrobenzimidazole* (*Compound 10*) (R = COSMe)

Compound 5 was condensed with methyl thiolochloroformate. When water and ether were added to the residue a solid precipitated. It was filtered off. The residue left on removal of the ether yielded yellow crystals melting at 140–156° (from methanol, then acetone–propan-2-ol) (Found: N, 4·8†. $C_{10}H_8ClN_3O_3S$ requires N, 4·9%). The solid which was filtered off was crystallised from acetone–propan-2-ol and gave pale yellow crystals, m.p. 172–174° (Found: N, 4·6†. $C_{10}H_8ClN_3O_3S$ requires N, 4·9%).

When the chloroformates were replaced in the above experiments by (dimethyl)- or (diethyl)-thiocarbamoyl chlorides or acetyl chloride only the starting material (Compound 5) was recovered from the crude reaction mixtures.

Preparation of potassium salts of diethyl phosphoro- and phosphono-thiolic and thiolothionic acids (II) (Compounds 11–14)

Potassium diethyl phosphorothiolothionate (Compound 11) (X = S; R^{IV} = OEt) was prepared according to the method of Pianka,[9] and potassium diethyl phosphorothiolate (Compound 12) (X = O; R^{IV} = OEt) by the method of Bolotova *et al.*[10] Potassium diethyl phosphonothiolothionate (Compound 13) (X = S; R^{IV} = Et) was prepared by neutralising *O*-ethyl ethylphosphonothiolothionic acid,[11] in acetone, with potassium carbonate; it was obtained as white prisms (from acetone), m.p. 202–204°. Potassium diethyl phosphonothiolate (Compound 14) (X = O; R^{IV} = Et) was obtained from *O*-ethyl ethylphosphonothiolic acid[12] as white prisms (from acetone), m.p. 168°.

Preparation of 2-(diethoxyphosphinothioylthioalkyl)benzimidazoles (III) (Compounds 15–20)

A solution of the chloromethylbenzimidazole (**I**) (0·01 mole) and the appropriate potassium salt (**II**) (0·01 mole) in 70 ml acetone was heated under reflux (Compounds 15, 17, 18, 20) or kept at room temperature for 24 h (Compounds 16, 19) and filtered, and the acetone was removed from the filtrate under reduced pressure (Scheme 1).

†Here and throughout the paper indicates analysis by way of nitro-groups after reduction with titanous chloride

*Petroleum refers to light petroleum, b.p. 60–80°

2-(*Diethoxyphosphinothioylthiomethyl*)*benzimidazole* (*Compound* 15) (X = S; R, R^{I}, R^{II}, R^{III} = H; R^{IV} = OEt)

This was obtained after reaction for 7·5 h and after the residue from the acetone had been dissolved in benzene, washed well with 2N-sodium carbonate, then with water, and dried over anhydrous sodium sulphate; the benzene was removed from the solution under reduced pressure and the residue was crystallised from ether (with the aid of charcoal), toluene, then ethyl acetate to yield (37%) white crystals, m.p. 112° (Found: P, 9·8. $C_{12}H_{17}N_2O_2PS_2$ requires P, 9·8%).

Compound 15 could also be prepared from 2-benzimidazolemethanethiol and diethyl phosphorochloridothionate. 2-Benzimidazolemethanethiol[13] (5·3 g) was dissolved in a solution of potassium hydroxide pellets (2·13 g) in methanol (70 ml). The methanol was removed under reduced pressure. The residue, in ethanol (50 ml), was added to diethyl phosphorochloridothionate[14] (6·1 g), in ethanol (10 ml), and the mixture heated under reflux for 1 h, cooled and filtered. The volatile components were removed under reduced pressure. The residue was dissolved in benzene (100 ml), washed with dilute aqueous sodium carbonate, then with water, and dried. The oil that remained after distillation of the solvent solidified on standing. The compound (1·5 g; 74%), m.p. 108–109·5°, gave no depression with Compound 15.

2-(*Diethoxyphosphinothioylthiomethyl*)-5(6)-*chlorobenzimidazole* (*Compound* 16) (X = S; R, R^{I}, R^{III} = H; R^{II} = Cl; R^{IV} = OEt)

In this case the residue was dissolved in ether, washed with aqueous potassium carbonate, then with water, and dried; the solvent was evaporated off and the residue was crystallised from toluene then petroleum to yield (55%) white crystals, m.p. 104–106° (Found: N, 10·3; P, 9·0; S, 18·6. $C_{12}H_{16}ClN_2O_2PS_2$ requires N, 10·1; P, 8·8; S, 18·3%).

2-(*Diethoxyphosphinothioylthiomethyl*)-5(6)-*nitrobenzimidazole* (*Compound 17*) (X = S; R, R^{I}, R^{III} = H; R^{II} = NO_2; R^{IV} = OEt)

The residue obtained after refluxing for 3 h was dissolved in benzene, washed well with 2·5% aqueous potassium carbonate, then water, and dried; on removal of the benzene a viscous orange oil, n_D^{30} 1·6215, was obtained and this was stirred with toluene to yield (78%) yellow prisms, m.p. 83–85° (Found: N, 3·8†; P, 8·9. $C_{12}H_{16}N_3O_4PS_2$ requires N, 3·9; P, 8·6%).

2-[1-(*Diethoxyphosphinothioylthio*)*ethyl*]-5(6)-*nitrobenzimidazole* (*Compound 18*) (X = S; R, R^{III} = H; R^{I} = Me; R^{II} = NO_2; R^{IV} = OEt)

After reflux for 1·5 h and condensation in ethyl methyl ketone, the residue was dissolved in benzene, washed with aqueous sodium carbonate, then water, and dried; the benzene was removed under reduced pressure and the residue solidified to yield (63%) pale yellow prisms, m.p. 86–88° (Found: N, 11·9; P, 8·1. $C_{13}H_{18}N_3O_4PS_2$ requires N, 11·2; P, 8·3%).

2-(*Diethoxyphosphinothioylthiomethyl*)-5(6)-*chloro*-6(5)-*nitrobenzimidazole* (*Compound 19*) (X = S; R, R^{I} = H; R^{II} = Cl; R^{III} = NO_2; R^{IV} = OEt)

This was prepared in the same way as Compound 16 and yielded (75%) pale yellow prisms, m.p. 91–93° (Found: P, 7·9. $C_{12}H_{15}ClN_3O_4PS_2$ requires P, 7·8%).

2-(*Diethoxyphosphinothioylthiomethyl*)-1-*methyl*-5-*nitrobenzimidazole* (*Compound 20*) (X = S; R = Me; R^{I}, R^{III} = H; R^{II} = NO_2; R^{IV} = OEt)

After reflux for 0·5 h, the residue was crystallised from propan-2-ol, then butanol to yield (69%) glistening yellow plates, m.p. 186·5–187° (Found: N, 11·1; P, 8·1; S, 16·7. $C_{13}H_{18}N_3O_4PS_2$ requires N, 11·2; P, 8·3; S, 17·1%).

2-(Diethoxyphosphinothioylthiomethyl)-1-acetylbenzimidazole (Compound 21) (III) (X = S; R = COMe; R^{I}, R^{II}, R^{III} = H; R^{IV} = OEt)

2-Chloromethylbenzimidazole (25 g) and acetic anhydride (60 ml) were heated on a steam bath for 3·5 h. The mixture was then kept at room temp. for 3 days, the volatile components were removed under reduced pressure, and the residue was extracted with boiling ligroin. The solid that separated on cooling (16·3 g) had m.p. 106–107° (from di-isopropyl ether, then carbon tetrachloride). Equimolar amounts of this solid and of potassium diethyl phosphorothiolothionate (Compound 11), in acetone, were kept at room temp. for 16 h, the mixture was filtered, the volatile components were removed from the filtrate under reduced pressure, and the residue was crystallised from acetone. The compound (69·5%) was obtained as white needles, m.p. 112·5° (Found: N, 7·7; P, 8·9; S, 17·9. $C_{14}H_{19}N_2O_3PS_2$ requires N, 7·8; P, 8·7; S, 17·9%).

2-(Diethoxyphosphinothioylthiomethyl)-1-(ethoxycarbonyl) benzimidazole (Compound 22) (III) (X = S; R = CO_2Et; R^{I}, R^{II}, R^{III} = H; R^{IV} = OEt)

To Compound 15 (6·4 g) and ethyl chloroformate (2·2 ml), in benzene, (50 ml) was added pyridine (1·6 g), in benzene (10 ml), and the mixture was heated under reflux for 16 h, washed well with dilute sulphuric acid, then with water. The benzene layer was dried and the benzene was distilled off under reduced pressure. The compound (6·3 g; 80·6%) was obtained as colourless prisms, m.p. 99–100° (from di-isopropyl ether) (Found: N, 7·7; P, 8·1; S, 16·7. $C_{15}H_{21}N_2O_4PS_2$ requires N, 7·2; P, 8·0; S, 16·5%). The phosphate obtained on condensing 2-chloromethyl-1-(ethoxycarbonyl) benzimidazole (**IV**; R = CO_2Et) and the potassium salt (Compound 11) also melted at 99–100°, and gave no depression with Compound 22.

2-(Diethoxyphosphinylthiomethyl)-5(6)-nitrobenzimidazole (Compound 23) (IX) (X = O; R^{I} = OEt)

This was prepared in the same way as Compounds 15–20.

After reflux for 3 h the residue was dissolved in benzene, washed with aqueous sodium carbonate, then with water, and dried with anhydrous sodium sulphate. The benzene was removed under reduced pressure and the residue was crystallised from benzene, then propan-2-ol to yield (45%) orange-yellow prisms, m.p. 127–129° (Found: N, 4·0†; P, 8·9. $C_{12}H_{16}N_3O_5PS$ requires N, 4·1; P, 9·0%).

Preparation of phosphinothioyl- or phosphinyl-thiomethyl -1-(alkoxy- or alkylthiolo-carbonyl) nitrobenzimidazoles (VIII) (Compounds 24–29)

These were prepared in the same way as Compounds 15–20, but were kept at room temp. for 24 h.

2-(*Diethoxyphosphinothioylthiomethyl*)-1-(*ethoxycarbonyl*) *nitrobenzimidazole* (*Compound 24*) (X = S; R = CO_2Et; R^1 = OEt)

The residue was dissolved in ether, washed with water, the ether was removed from the solution, and the residue was crystallised from di-isopropyl ether to yield a mixture of pale yellow needles and prisms melting at 84–97°. On fractional crystallisation from methanol pale yellow needles, m.p. 103–104°, were obtained as the first crop (Found: N, 9·7; P, 7·0%) and pale yellow rectangular prisms, m.p. 94–95° (from benzene–petroleum, then di-isopropyl ether) (Found: N, 9·6; P, 7·1%) as the second crop. Mixed m.p. of first and second crops showed a strong depression ($C_{15}H_{20}N_3O_6PS_2$ requires N, 9·7; P, 7·2%).

2-[*Ethoxy(ethyl)phosphinothioyl- and ethoxy(ethyl)phosphinylthiomethyl*]-1-(*ethoxycarbonyl*) *nitrobenzimidazoles* (*Compounds* 25, 26) (X = S or O; R = CO_2Et; R^1 = Et)

The residue was dissolved in benzene, washed with 2N-sodium carbonate, then with water, and dried; the benzene was removed under reduced pressure, but the residual oils could not be crystallised. Compound 25 (64%) was a pale brown oil, n_D^{23} 1·6002 (Found: N, 3·2†. $C_{15}H_{20}N_3O_5PS_2$ requires N, 3·4%). Compound 26 (69%) was a dark red viscous oil (Found: N, 3·5†; P, 7·8. $C_{15}H_{20}N_3O_6PS$ requires N, 3·5; P, 7·7%).

2-(*Diethoxyphosphinothioylthiomethyl*)-1-(*isopropoxy- and butoxy-carbonyl*) *nitrobenzimidazoles* (*Compounds 27, 28*) (X = S; R = CO_2 Pr^i or CO_2 Bu; R^1 = OEt)

These were prepared in the same way as Compound 24, and when crystallised from methanol yielded off-white prisms (89%), m.p. 88–90°, and white prisms (30%), m.p. 102–106°, respectively (Found: N, 3·1†; P, 7·4 and N, 3·0†; P, 7·0. $C_{16}H_{22}N_3O_6PS_2$ requires N, 3·1; P, 6·9%. $C_{17}H_{24}N_3O_6PS_2$ requires N, 3·0; P, 6·7%).

2-(*Diethoxyphosphinothioylthiomethyl*)-1-(*methylthiolocarbonyl*) *nitrobenzimidazole* (*Compound 29*) (X = S; R = COSMe; R^1 = OEt)

The residue consisted of an oil and a solid; ether was added and the mixture was washed with 1% aqueous sodium bicarbonate. The insoluble solid was filtered off, the ether layer was washed with water and dried, and the ether was distilled off. The residue was crystallised from propan-2-ol yielding (60%) bright yellow prisms, which were then recrystallised from methanol. The compound (40%) was obtained as pale yellow prisms, m.p. 112–116° (Found: N, 3·0†; P, 7·2. $C_{14}H_{18}N_3O_5PS_3$ requires N, 3·2; P, 7·1%).

2-(Diethoxyphosphinothioylthiomethyl)-1-(methoxymethyl) nitrobenzimidazole (Compound 30) (VIII) (X = S; R = CH_2OMe; R^1 = OEt)

To Compound 17 (3·6 g), in sodium-dried ether (60 ml), were added pyridine (0·79 g), then chloromethyl methyl ether (0·8 g), in sodium-dried ether (10 ml). The mixture was kept for 4 days at room temp. Water was then added. The ether layer was separated, washed with water, then dried, and the volatile components were then removed from it. The residue (3·3 g; 82·5%) was obtained as a brown oil, n_D^{22} 1·6082 (Found: N, 3·6†. $C_{14}H_{20}N_3O_5PS_2$ requires N, 3·6%).

Results and Discussion

2-Chloromethyl-5(6)-nitrobenzimidazole (Compound 5) was prepared in 89% yield by the nitration of 2-chloromethylbenzimidazole hydrochloride. Ozegowski *et al.*[6] obtained a yield of only 65% on nitrating the base. They also prepared 2-chloromethyl-1-methyl-5-nitrobenzimidazole (Compound 3) by cyclisation of 2-methylamino-5-nitro-α-chloroacetanilide. This compound was readily prepared by the chlorination of 2-hydroxymethyl-1-methyl-5-nitrobenzimidazole. On condensation with potassium salt (Compound 11) it yielded phosphate (Compound 20).

2-Chloromethylbenzimidazoles with no substituents in the benzene ring gave phosphates with sharply defined melting points. Thus, 2-chloromethylbenzimidazole (Compound 1) and the potassium salt (Compound 11) gave phosphate (Compound 15) identical with that obtained from 2-benzimidazolemethanethiol and diethyl phosphorochloridothionate. 2-Chloromethyl-1-(ethoxycarbonyl)benzimidazole and potassium salt (Compound 11) gave phosphate (Compound 22), which was identical with that obtained from the ring-unsubstituted phosphate (Compound 15) and ethyl chloroformate.

2-Chloromethylbenzimidazoles with substituents in the benzene ring only, also gave phosphates with sharply defined melting points. 2-Chloromethyl-5(6)-nitrobenzimidazole (Compound 5) with salt (Compound 11) or salt (Compound 12) gave phosphates (Compound 17 or 23). With potassium salt (Compound 11), 2-(1-chloroethyl)-5(6)-nitrobenzimidazole (Compound 4), 5(6)-chloro-2-chloromethylbenzimidazole (Compound 2), and 5(6)-chloro-2-chloromethyl-6(5)-nitrobenzimidazole (Compound 6) gave the phosphates (Compounds 18, 16 and 19), respectively.

However, phosphates (Compounds 24–29) obtained by the condensation of 2-chloromethyl-5(6)-nitrobenzimidazole (V) with a chloroformate, then with a potassium salt, or phosphate (Compound 30) obtained by the condensation of the ring-substituted phosphate (Compound 17) and chloromethyl methyl ether, melted over an extended temperature range or were oils, indicative of mixtures of isomers.

The Murphy Chemical Co. Ltd.,
Wheathampstead,
St. Albans, Herts.

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References

1. Wright, J. B., *Chem. Rev.*, 1951, **48**, 397
2. Phillips, M. A., *J. chem. Soc.*, 1928, p. 2393
3. Lettré, H., Fritsch, W., & Porath, J., *Chem. Ber.*, 1951, **84**, 719
4. Bloom, A., & Day, A. R., *J. org. Chem.*, 1939, **4**, 14
5. Joseph, L., & Julca, J., *J. org. Chem.*, 1962, **27**, 1101
6. Ozegowski, W., Krebs, D., & Wunderwald, M., *J. prakt. Chem.*, 1963, **20**, 166
7. Siegart, W. R., & Day, A. R., *J. Am. chem. Soc.*, 1957, **79**, 4391
8. Grimaldi, A. M., & Day, A. R., *J. org. Chem.*, 1962, **27**, 227
9. Pianka, M., *J. Sci. Fd Agric.*, 1967, **18**, 63
10. Bolotova, G. L., Kotova, G. G., Zimina, K. L., & Isagulyants, V. J., *Izv. vyssh. ucheb. Zaved.*, 1965, **8**, 62
11. Chupp, J. P., & Newallis, P. H., *J. org. Chem.*, 1962, **27**, 3832
12. Hoffmann, F. W., Kagan, B., & Canfield, J. H., *J. Am. chem. Soc.*, 1959, **81**, 148
13. Fedorov, B. P., & Mamedov, R. M., *Izv. Akad. Nauk SSSR, Otdel. khim. Nauk*, 1962, p. 1626
14. Mastin, T. W., Norman, G. R., & Weilmuenster, E. A., *J. Am. chem. Soc.*, 1945, **67**, 1662

ORGANOPHOSPHORUS COMPOUNDS

V.*—Structure and activity of certain 2-[diethoxy or ethoxy (ethyl) phosphinylthio- and diethoxy or ethoxy (ethyl) phosphinothioylthio-methyl] benzimidazoles

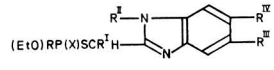
By M. PIANKA

Thirteen substituted 2-(diethoxyphosphinothioylthiomethyl) benzimidazoles, a 2-(diethoxyphosphinylthiomethyl) benzimidazole, an [ethoxy (ethyl)-phosphinothioylthio]- and [phosphinylthio]-methylbenzimidazole were tested for activity against *Aphis fabae*, *Tetranychus telarius*, *Musca domestica* and *Plutella maculipennis* (Curtis). For high contact activity a nitro-group on the benzene nucleus was required. Substitution on the nitrogen atom of the nitrobenzimidazole radical had a variable effect on activity; the thiolomethylcarbonyl group suppressed it. Substitution by a methyl group on the carbon of the S-methyl reduced activity. Replacement of P(S) by P(O) in the diethoxy-compounds greatly enhanced systemic activity.

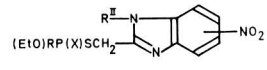
Introduction

Benzimidazoles are known to possess biological activity. Some antagonise the action of purines in living cells and some viruses, others inhibit the incorporation of amino-acids into bacteria,[2] and others have an effect on insects,[3] mites,[4] plant growth,[5] and on enzyme activity.[6]

It became of interest to combine the phosphate and benzimidazole toxiphores with the object of studying the biological activity of the resulting phosphates (**I, II**).



(I)



(II)

Experimental

The preparation of the compounds listed in Table I was described in Part IV.[1] The position of the nitro-group in Compounds 24–30 (see Part IV) was not determined, since the chief interest was the study of the effect of the introduction of this electron-attracting group on activity.

Formulation

The compounds were formulated as 5% w/v (Compounds 16, 18–22, 24) or 10% w/v (Compounds 15, 17, 23, 25–30) emulsifiable concentrates in acetone and the emulsifier Lissapol NX (a polyethylene glycol ether), and their activities were tested by various methods.

In all the spraying, dipping and leaf painting tests for insecticidal, but not acaricidal, activity, 250 ppm of a non-ionic wetter were added to all the treatments to aid wetting of the plant surface.

Contact activity

Contact aphicidal activity

These tests were carried out as previously described.[7]

Contact acaricidal activity

These tests were carried out as previously described on *Tetranychus telarius* mites not resistant to organophosphorus compounds.[7] With certain compounds tests were carried out by the previously described technique on *Tetranychus telarius* mites resistant to organophosphorus compounds.[8]

Contact activity against Musca domestica

Twenty adult house-flies up to 3 days old were anaesthesised with carbon dioxide and placed in a cylinder with a muslin bottom. The top of the cylinder was covered with muslin held with an elastic band. The cage was then placed on a filter paper in a funnel fitted to a suction pump. The diluted toxicant was then poured, with suction applied, over the flies through the muslin cover. After treatment the cage was placed on a cotton-wool pad soaked in a 10% sugar solution in a Petri dish, and kept at 80°F and 60% r.h. The assessments were carried out after 48 h, and the percentage kill was calculated, with the correction for natural mortality, by Abbot's formula.

* Part IV: Preceding paper

TABLE I

Compounds used in the tests

Compound	Name of compound		X	R	R^{I}	R^{II}	R^{III}	R^{IV}
15	2-(Diethoxyphosphinothioylthiomethyl) benzimidazole	(I)	S	EtO	H	H	H	H
16	2-(Diethoxyphosphinothioylthiomethyl)-5(6)-chlorobenzimidazole	(I)	S	EtO	H	H	Cl	H
17	2-(Diethoxyphosphinothioylthiomethyl)-5(6)-nitrobenzimidazole	(I)	S	EtO	H	H	NO_2	H
18	2-[1-(Diethoxyphosphinothioylthio) ethyl]-5(6)-nitrobenzimidazole	(I)	S	EtO	Me	H	NO_2	H
19	2-(Diethoxyphosphinothioylthiomethyl)-5(6)-chloro-6(5)-nitrobenzimidazole	(I)	S	EtO	H	H	Cl	NO_2
20	2-(Diethoxyphosphinothioylthiomethyl)-1-methyl-5-nitrobenzimidazole	(I)	S	EtO	H	Me	NO_2	H
21	2-(Diethoxyphosphinothioylthiomethyl)-1-acetylbenzimidazole	(I)	S	EtO	H	COMe	H	H
22	2-(Diethoxyphosphinothioylthiomethyl)-1-(ethoxycarbonyl) benzimidazole	(I)	S	EtO	H	CO_2Et	H	H
23	2-(Diethoxyphosphinylthiomethyl)-5(6)-nitrobenzimidazole	(I)	O	EtO	H	H	NO_2	H
24	2-(Diethoxyphosphinothioylthiomethyl)-1-(ethoxycarbonyl) nitrobenzimidazole	(II)	S	EtO		CO_2Et		
25	2-[Ethoxy(ethyl)phosphinothioylthiomethyl]-1-(ethoxycarbonyl) nitrobenzimidazole	(II)	S	Et		CO_2Et		
26	2-[Ethoxy(ethyl)phosphinylthiomethyl]-1-(ethoxycarbonyl) nitrobenzimidazole	(II)	O	Et		CO_2Et		
27	2-(Diethoxyphosphinothioylthiomethyl)-1-(isopropoxycarbonyl) nitrobenzimidazole	(II)	S	EtO		CO_2Pr^{i}		
28	2-(Diethoxyphosphinothioylthiomethyl)-1-(butoxycarbonyl) nitrobenzimidazole	(II)	S	EtO		CO_2Bu		
29	2-(Diethoxyphosphinothioylthiomethyl)-1-(methylthiolocarbonyl) nitrobenzimidazole	(II)	S	EtO		COSMe		
30	2-(Diethoxyphosphinothioylthiomethyl)-1-(methoxymethyl) nitrobenzimidazole	(II)	S	EtO		CH_2OMe		

Contact and stomach activity against Plutella maculipennis *(Curtis)*

Petioles of four small rape leaves were inserted through holes bored in a cork fitted into a water-filled specimen tube, then surrounded with non-absorbent cotton-wool plugs to prevent evaporation through the gap between the holes and the petioles. The leaves were then dipped in the dilute toxicant and allowed to drain and dry. The tubes were then placed in a Petri dish containing a layer of several filter papers, and a lamp glass was placed on the dish to form a cage. Plasticine was placed on the outer bottom ring of the lamp glass to prevent escape of caterpillars. On each leaf were placed twenty 2nd or 3rd instar larvae of the diamond back moth, and the lamp glass was covered with a muslin square. The plants were kept at 75°F and 60% r.h. The assessments were then carried out after 48 h, and the percentage kill was calculated, with the correction for natural mortality, by Abbot's formula.

Systemic activity

Systemic aphical activity by spraying of broad-bean plants

The tests were carried out as described previously.[7] The toxicants were tested at a dilution of 300 and 100 ppm.

Systemic aphicidal activity by spraying of sugar-beet plants

The activity of several compounds was tested on sugar-beet plants as described previously.[7]

Systemic aphicidal activity on broad-bean plants by uptake by the roots from culture solutions

Three broad-bean plants were removed from the soil and their roots were washed and drained. Cotton wool was wrapped round the base of each stem and the stem was placed through a split cork fitted in a jar. The roots of the plant were immersed in the jar containing the toxicant in culture solution. About fifty aphids (*Aphis fabae*) were allowed to transfer from infested plants on to each plant kept at 75° F and 60% r.h., and after two days assessments were carried out. The solutions in the jars were replaced by pure culture solution. The plants were then re-infested and assessments were carried out 4 days later (6 days from start of test), again re-infested and re-assessed 7 days later (13 days from start of test).

Systemic aphicidal activity on broad-bean plants by soil application

For each treatment three broad-bean plants were used. The dilution of the toxicant (20 ml) was run from a pipette on to the soil around the base of the plants. The plants were artificially infested with *Aphis fabae*. Assessments were carried out 2 days later, the plants were re-infested and assessed after a further 2 days, and this was repeated.

Systemic aphicidal activity on broad-bean plants by stem painting

For each treatment three broad-bean plants without side shoots were used. Cotton-wool collars were placed round the base of the stem. The diluted toxicant (0·2 ml of the required concentration to which 1000 ppm of wetter were added) was dropped from a pipette on to the stem about one inch above the cotton-wool. The plants were artificially infested 1 day after treatment and assessed 2 days after infestation.

Translaminar aphicidal activity on sugar-beet plants

For each treatment three sugar-beet plants at the six-leaf stage were used. The diluted toxicant was painted on the upper surfaces of the leaves thrice at three-hour intervals. Each plant was infested with 50 aphids (*Aphis fabae*) on the underside of the leaves and kept at 75° F and 60% r.h. The assessments were carried out 2 days after infestation.

Standards

The following compounds were used as standards in the systemic tests: demeton-methyl, a mixture of 2-(ethylthio) ethyl dimethyl phosphorothionate and *S*-[2-(ethylthio) ethyl] dimethyl phosphorothiolate; dimethoate, dimethyl *S*-(*N*-methylcarbamoylmethyl) phosphorothiolothionate; dimefox, *N,N,N′,N′*-tetramethylphosphorodiamidic fluoride; schradan, i.e. bis *N,N,N′,N′*-tetramethylphosphorodiamidic anhydride.

Mammalian toxicity

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

The finely ground compounds were dissolved in propylene glycol (Compounds 17 and 23), or suspended in distilled water with 0·5% Tween 80 (Compound 24). The concentrations varied between 6 and 180 mg/ml. Forty to 80 I.C.I. male mice (bodyweight range 26–31 g) and 24 to 30 male Wistar rats (bodyweight 190–260 g) were used per determination (for Compound 24 female Wistar rats were used). The mortality was observed after 7 days. Median lethal doses are presented in Table VIII.

Results and Discussion

Contact activity

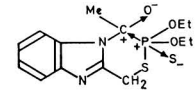
Effect on activity of substitution in the benzene ring

Compounds 15 and 22, which were not substituted in the benzene ring had low activity (Table II). Substitution in position 5 with chlorine (Compound 16) enhanced activity only slightly but substitution with a nitro-group increased it markedly (Compounds 15 and 17; 16 and 19; 22 and 24). This observation is in line with the observed increase in toxicity of diethyl aryl phosphorothionates in the approximate order $H < Cl < NO_2$ and the increasing electron-withdrawing effect of the substituents.[9] The strongly electron-withdrawing nitro-group on the benzenoid benzimidazole[10] enhances the electrophilic character of the phosphorus atom and thus the formation of the enzyme-inhibitor complex.[11]

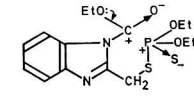
Effect on activity of substitution in the 1-position

Substitution with an acetyl group (Compound 21) had little effect on contact aphicidal activity, enhanced slightly the activity against spider mites and greatly enhanced the activity against *Musca* and *Plutella* (Table II). This increase in activity may be due to the polarisation of the carbonyl bond which renders the carbon atom positive thus enhancing the electrophilic character of the phosphorus atom (**III**) and facilitating phosphorylation of the enzyme.

In the 1-(alkoxycarbonyl)-substituted compound (22) the oxygen atom was capable of electron release. This would reduce the electron-withdrawing effect of the carbonyl group on the phosphorus atom (**IV**) and stabilise the compound to hydrolysis; Compound 22 has low activity. Hydrolysis of the ethoxycarbonyl group by carboxyesterases is also feasible,[12] which, after the expected loss of carbon dioxide, would yield Compound 15. It is significant that diethyl 4-(ethoxycarbonyl)phenyl phosphorothionate also has low activity.[9]



(III)



(IV)

TABLE II

Contact activities against *Aphis fabae*, *Tetranychus telarius*, *Musca domestica* and *Plutella maculipennis* of [diethoxy or ethoxy (ethyl) phosphinylthio- and diethoxy or ethoxy (ethyl) phosphinothioylthio-methyl] benzimidazoles

Compound	X	R	R^I	R^{II}	R^{III}	R^{IV}	*Aphis fabae* Kill (%) at ppm					*Tetranychus telarius** Kill (%) at ppm					*Musca domestica* Kill (%) at ppm		*Plutella maculipennis* Kill (%) at ppm		
							100	30	10	3	1	100	30	10	3	1	300	100	300	100	30
Effect of nitro-group on activities of 2-(diethoxyphosphinothioylthiomethyl) benzimidazoles																					
15 (I)	S	EtO	H	H	H	H	89	41	13	5	–†	68	35	6	–	–	3	–	0	–	–
17 (I)	S	EtO	H	H	NO_2	H	100	100	98	65	34	97	93	67	39	–	91	40	60	–	–
												(61	39	—	–	–)					
16 (I)	S	EtO	H	H	Cl	H	84	75	38	–	–	61	44	26	–	–	5	–	20	–	–
19 (I)	S	EtO	H	H	Cl	NO_2	97	94	92	34	25	97	75	56	–	–	55	–	100	40	20
22 (I)	S	EtO	H	CO_2Et	H	H	29	13	5	–	–	59	29	21	–	–	25	–	55	–	–
24 (I)	S	EtO	H	CO_2Et	(H and NO_2)		100	99	95	90	82	100	95	80	49	–	65	–	95	45	25
												(73	39	41	–	–)					
Effect of 1-substitution on activities of 2-(diethoxyphosphinothioylthiomethyl) benzimidazoles																					
15 (I)	S	EtO	H	H	H	H	89	41	13	5	–	68	35	6	–	–	3	–	0	–	–
21 (I)	S	EtO	H	COMe	H	H	81	41	9	–	–	87	65	45	–	–	80	–	100	87	–
22 (I)	S	EtO	H	CO_2Et	H	H	29	13	5	–	–	59	29	–	–	–	25	–	55	–	–
Effect of 1-substitution on activities of 2-(diethoxyphosphinothioylthiomethyl) nitrobenzimidazoles																					
17 (I)	S	EtO	H	H	NO_2	H	100	100	98	65	34	97	93	67	39	–	91	40	60	–	–
20 (I)	S	EtO	H	Me	NO_2	H	96	93	94	80	88	100	98	94	82	59	30	–	87	85	75
24 (I)	S	EtO	H	CO_2Et	(H and NO_2)		100	99	95	90	82	100	95	80	49	–	65	–	95	45	25
27 (I)	S	EtO	H	CO_2Pr^i	(H and NO_2)		100	100	81	–	–	99	96	87	–	–	4	–	55	–	–
28 (I)	S	EtO	H	CO_2Bu	(H and NO_2)		100	98	89	–	–	99	93	82	–	–	0	–	15	–	–
29 (I)	S	EtO	H	COSMe	(H and NO_2)		14	20	18	–	–	47	12	14	–	–	0	–	25	–	–
												(27	12	5	–	–)					
30 (I)	S	EtO	H	CH_2OMe	(H and NO_2)		100	85	91	–	–	100	93	77	–	–	0	–	35	10	10
Activities of 2-[diethoxy- and ethoxy (ethyl) phosphinylthio- and diethoxy and ethoxy (ethyl) phosphinothioylthio-methyl] nitrobenzimidazoles																					
17 (I)	S	EtO	H	H	NO_2	H	100	100	98	65	34	97	93	67	39	–	91	40	60	–	–
23 (I)	O	EtO	H	H	NO_2	H	100	100	94	66	21	95	69	36	–	–	97	25	55	–	–
24 (I)	S	EtO	H	CO_2Et	(H and NO_2)		100	99	95	90	82	100	95	80	49	–	65	–	95	45	25
25 (I)	S	Et	H	CO_2Et	(H and NO_2)		97	96	90	90	57	100	100	99	98	83	–	–	98	90	63
												(89	63	42	–	–)					
26 (I)	O	Et	H	CO_2Et	(H and NO_2)		100	100	89	39	23	100	100	100	86	–	–	–	84	76	32
												(93	80	76	65	82)					
Effect of alkyl substitution in the methylene chain of 2-(diethoxyphosphinothioylthiomethyl)-5(6)-nitrobenzimidazole																					
17 (I)	S	EtO	H	H	NO_2	H	100	100	98	65	34	97	93	67	39	–	91	40	60	–	–
18 (I)	S	EtO	Me	H	NO_2	H	100	91	65	–	–	100	56	31	–	–	0	–	85	–	–

* *Tetranychus telarius* mites not resistant to organophosphorus compounds; in brackets is given activity against *Tetranychus telarius* resistant to organophosphorus compounds † Not tested at this concentration

All the nitro-substituted compounds (Table II) were active against aphids and spider mites, with the surprising exception of the 1-(thiolomethylcarbonyl)-substituted compound (29). It is not possible to explain its low activity on the basis of the electron effect of the SMe group. Since on hydrolysis Compound 29 would be expected to yield the highly active Compound 17 one may assume that hydrolysis does not occur. Perhaps the low activity of Compound 29 is due to its inability to penetrate to the vital sites of action or to fit the enzyme. Reduction in acaricidal activity has also been observed when an oxygen was replaced by a sulphur atom in the carbonate chain of 2-alkyl-4,6-dinitrophenols.[8,13] 1-Substitution with a methyl or ethoxycarbonyl group (Compounds 20, 24; Table II) enhanced the activity, compared with Compound 17, against aphids, spider mites and *Plutella*, but reduced its activity against *Musca*. Increase in the alkyl chain of the alkoxycarbonyl group (Compounds 24, 27, 28) had little effect on aphicidal or acaricidal activity, but reduced activity against *Musca* and *Plutella*. 1-Substitution with a methoxymethyl group (Compound 30) had little effect on aphicidal or acaricidal activity, but reduced the activity of Compound 17 against *Musca* and *Plutella*.

Effect of replacing S by O on the phosphorus atom

The P(O) Compound (23; Table II) was as active against aphids, *Musca* and *Plutella* as its P(S) analogue (Compound 17), but less active against spider mites. However, in the ethoxy (ethyl) series the P(S) compound (25) was more active against aphids, non-resistant spider mites and *Plutella*, whereas the P(O) analogue (Compound 26) was more active against resistant spider mites. The ethoxy (ethyl) compound (25) was more active than its diethoxy analogue (24) against spider mites and *Plutella*.

Effect of methyl substitution in the S.CH_2 group

Substitution of the methylene group with a methyl group resulted in a reduction in activity (Compounds 17 and 18; Table II). The reduction may be due to electronic and steric influences of the methyl substituent having the effect of stabilising Compound 18 to hydrolysis.[14]

Systemic activity

Tables III–VII present systemic aphicidal tests with several compounds. Of nine benzimidazole phosphates possessing high contact aphicidal activity only the P(O) derivative (Compound 23) showed activity on new untreated leaves of broad-bean plants (Table III) or sugar-beet plants (Table IV) the aerial parts of which had been treated. Some P(S) compounds (17 and 24), though of low activity in the above systemic tests, were active when tested on broad-bean plants by root uptake from culture solutions (Table V), but not from soil (Table VI). Compound 24 and its ethoxy (ethyl) analogue (Compound 25) were not translocated from the stem into the leaves of broad-bean plants, but were translocated from the upper- to the under-surfaces of sugar-beet leaves (Table VII).

TABLE III

Systemic aphicidal activity by spraying of broad-bean plants

Compound	Substituent in position			Kill (%) of *Aphis fabae* at ppm							
				I Test		II Test		III Test		IV Test	
	1	5 (6)	6 (5)	300	100	300	100	300	100	300	100
17	H	NO_2	H	35	4	65	24	—	—	—	—
19	H	Cl	NO_2	—	—	—	—	22	1	—	—
20	Me	5-NO_2	H	—	—	—	—	3	1	—	—
23	H	NO_2	H	89	—	77	—	—	0	—	—
24	CO_2Et	(H and NO_2)		26	8	—	—	—	—	—	—
25	CO_2Et	(H and NO_2)		—	—	—	—	0	0	19	—
26	CO_2Et	(H and NO_2)		—	—	1	0	—	—	13	—
27	CO_2Pr^i	(H and NO_2)		—	—	2	1	—	—	—	—
28	CO_2Bu	(H and NO_2)		—	—	2	1	—	—	—	—
29	COSMe	(H and NO_2)		—	—	0	0	—	—	—	—
Demeton-methyl				100	100	96	73	98	76	100	93

TABLE IV

Comparison of systemic aphicidal activity by spraying of sugar-beet and broad-bean plants

	Systemic activity against *Aphis fabae* by spraying of				
	Sugar-beet plants		Broad-bean plants Kill (%) at ppm		
	1000	300	300 I Test	300 II Test	100 III Test
Compound 23, 2-(Diethoxyphosphinylthiomethyl)-5(6)-nitrobenzimidazole	100	71	89	77	0
Demeton-methyl	100	95	100	96	76
Dimethoate	100	89	—	—	—

TABLE V

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

Compound	Substituent in position			I Test Kill (%) at ppm									II Test Kill (%) at ppm					
				300			100			30			30		10		3	
	1	5 (6)	6 (5)	D*	E†	F‡	D*	E†	F‡	D*	E†	F‡	D*	E†	D*	E†	D*	E†
17	H	NO_2	H	74	96	79	48	90	81	11	61	54	36	62	13	31	1	5
19	H	Cl	NO_2										6	10				
20	Me	5-NO_2	H										2	7				
23	H	NO_2	H	99	100	99	100	100	99	98	99	30	100	95	94	78	79	49
24	CO_2Et	(H and NO_2)		97									4	9				
25	CO_2Et	(H and NO_2)		58	2													
26	CO_2Et	(H and NO_2)		100	99	99	99	94	86	74	65	0	59	51				
27	CO_2Pr^i	(H and NO_2)											0	1				
28	CO_2Bu	(H and NO_2)											45	77				
Demeton-methyl				100	100	100	100	100	100	100	100	100	100	100	100	100	100	100

* After 2 days † After 6 days ‡ After 13 days

TABLE VI

Systemic aphicidal activity on broad-bean plants by soil application

Compound	Substituent		I Test Kill (%) at ppm						II Test Kill (%) at 500 ppm			III Test Kill (%) at 500 ppm		
				500			250							
	R^{II}	Benzene ring	A*	B†	C‡	A*	B†	C‡	A*	B†	C‡	A*	B†	C‡
17	H	NO_2							0	4	6	1	41	68
23	H	NO_2							94	88	78	82	91	52
24	CO_2Et	NO_2	11	5	13									
25	CO_2Et	NO_2	7	9	7				1	1	1			
26	CO_2Et	NO_2	5	16	2									
Dimefox			99	87	78	99	51	47	94	89	93	100	100	100
Demeton-methyl												100	100	100

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

TABLE VII

Systemic aphicidal activity on broad-bean plants by stem painting and on sugar-beet plants by leaf painting

Compound	Substituent		Broad-bean plants Kill (%) at concentrations (%) of toxicant					Sugar-beet plants Kill (%) at ppm of toxicant				
	R^{II}	Benzene ring	2	1	0·5	0·25	0·125	500	100	50	25	12·5
23	H	NO_2	100	100	100	99	93			100	99	96
24	CO_2Et	NO_2		29				100				
25	CO_2Et	NO_2		3				100				
26	CO_2Et	NO_2		0				22				
Dimefox			69	6	3							
Demeton-methyl			100	100	100	100	99		100	100	100	97
Schradan					0	2	0					
Dimethoate										86	63	50

TABLE VIII

Mammalian toxicity of certain diethoxyphosphoromethylbenzimidazoles

Compound	X	R	R^{I}	R^{II}	R^{III}	R^{IV}	Acute oral LD_{50} mg/kg	
							Mice	Rats
17 (I)	S	EtO	H	H	NO_2	H	245	92
24 (I)	S	EtO	H	CO_2Et	(H and NO_2)		2650	88
23 (I)	O	EtO	H	H	NO_2	H	102	31

The diethoxy P(O) compound (23) was highly systemic. It was taken up by the roots from culture solution (Table V) and from the soil (Table VI) and by the stem of broad-bean plants (Table VII), and translocated to the leaves. It also moved across the leaves of sugar-beet plants (Table VII). The 1-(ethoxycarbonyl)-substituted ethoxy(ethyl) analogue (Compound 26) which had lower contact aphicidal activity was also less active systemically. It was not taken up from the soil by roots (Table VI) or translocated from the stem of broad-bean plants (Table VII). It had little translaminar activity in the sugar-beet test.

Demeton-methyl was highly active by leaf, root and stem application, dimefox was highly active by soil application, but not by stem application. Schradan was not translocated from the stem.

Mammalian toxicity

Numerous examples exist of increase of toxicity when P(S) has been replaced by P(O). Mammalian toxicity is generally increased by 2 to 10 times.[9] The P(O) compound (23) was about 2–3 times more toxic than the P(S) analogue (Compound 17) (Table VIII). The effect of the 1-substituent CO_2Et is interesting: though it had little effect on the toxicity to rats, it reduced the toxicity to mice by about 10 times (Compounds 17 and 24). It may be that the (ethoxycarbonyl) group of Compound 24 is hydrolysed in the rat and, on losing carbon dioxide, the compound degrades to Compound 17. Alternatively, the reduced toxicity to mice may be due to poor absorption of Compound 24.

Acknowledgments

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The Murphy Chemical Co. Ltd.,
Wheathampstead,
St. Albans, Herts.

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References

1. Pianka, M., & Edwards, J. D., *J. Sci. Fd Agric.*, 1968, **19,**
2. Albert, A., 'Heterocyclic chemistry', 1959, p. 192 (University of London: The Athlone Press)
3. Berim, N. G., & Efros, A. M. *Zap. Leningr. Selskokhoz. Inst.*, 1962, **87,** 19
4. Saggers, D. T., & Clark, M. L., *Nature, Lond.*, 1967, **215,** 275
5. Burton, D. E., Lambie, A. J., Ludgate, J. C. L., Newbold, G. T., Percival, A., & Saggers, D. T., *Nature, Lond.*, 1965, **208,** 116
6. Pavlov, V. S., *Zap. Leningr. Selskokhoz. Inst.*, 1963, **90,** 27
7. Pianka, M., & Polton, D. J., *J. Sci. Fd Agric.*, 1965, **16,** 330
8. Pianka, M., *J. Sci. Fd Agric.*, 1966, **17,** 47
9. Metcalf, R. L., 'Organic insecticides', 1955, p. 292 (New York: Interscience Publishers, Inc.)
10. Fries, K., *Annalen Chem.*, 1927, **454,** 191
11. Pianka, M., *J. Sci. Fd Agric.*, 1957, **8,** 393
12. O'Brien, R. D., 'Toxic phosphorus esters', 1960, p. 116 (New York: Academic Press)
13. Pianka, M., & Edwards, J. D., *J. Sci. Fd Agric.*, 1967, **18,** 355
14. Pianka, M., *J. Sci. Fd Agric.*, 1967, **18,** 63

NONYL ALCOHOL VAPOUR IN POTATO STORES DURING SPROUT SUPPRESSION

II.*—Physical factors in the uptake of nonyl alcohol vapour by potato tubers

By I. E. CURRAH and D. F. MEIGH

A study was made of some physical factors which influence the uptake by potato tubers of nonyl alcohol, used for sprout suppression in commercial storage. The saturation concentration of nonyl alcohol vapour in air was determined over the relevant temperature range. Investigation of the interactions between potato tubers and mixtures of air, nonyl alcohol vapour and water vapour showed that the tubers reached equilibrium with the alcohol vapour most rapidly in dry air at the higher of two temperatures investigated. The weight of alcohol taken up by the tuber was greater in dry air and was proportional to the square of the applied alcohol vapour concentration. A sudden rise in humidity caused a rapid release of some alcohol from a treated tuber, while a drop in humidity caused a rather slower uptake. The results suggest that part of the nonyl alcohol is absorbed by the bulk of the potato and part adsorbed on the surface of the skin. The relevance of the results to commercial potato storage practice is discussed.

Introduction

A preliminary investigation has been made of the nonyl alcohol vapour concentrations found in a commercial potato store where nonyl alcohol (3, 5, 5-trimethylhexanol-1) is used as a sprout suppressant.[1] It was found that the way in which the alcohol vapour was taken up and released was not entirely explicable in terms of changes in temperature or vapour concentration. In the rest periods between alcohol applications, when ventilation was stopped, the vapour concentration in the air of the stack increased to a level which was often in excess of that originally applied. Judging by the smell, the alcohol was also released from treated tubers on humid days.

A more detailed study was therefore made of the relationship between amount of alcohol in the tuber and in the surrounding air under varied conditions of ventilation and humidity. Since it is difficult to establish close control of the physical environment of large masses of potatoes, experiments were made with pairs of tubers. This report is concerned with variation with temperature and humidity of the saturation concentration of alcohol vapour in air and the interactions between tubers and mixtures of air, nonyl alcohol vapour and water vapour.

Experimental

Potatoes

Tubers of the variety Arran Comet, grown on a medium loam soil above calcareous Lower Greensand deposits in Kent, were lifted mechanically under dry conditions on 1 July 1966 when the skins were partly set. The tubers were placed on trays and held at a temperature of 10° for two weeks, then washed rapidly, dried and sorted. Damaged tubers were discarded and the sound ones were arranged in pairs, of weight ~125 g. The pairs of tubers were then allotted at random to the experimental treatments and stored at 10° for one month before the experiment commenced. At that time sprout growth was visible on a few tubers, and by the end of the experiment, five weeks later, most tubers carried sprouts 2–3 mm in length. The weight, dimensions and surface area of the chosen tubers were determined before use.

Measurement of the surface area of tubers

The open ends of two small beakers (5 ml) were placed one over each end of the tuber and the assembly was held together by a flat rubber band passing longitudinally around the beakers and the potato. A knot was tied at the end of a length of lightweight twin microphone cable (2·5 mm wide; Radiospares Ltd.) and anchored under the edge of one beaker so that the cable issued from the lip. The cable was then wound round the tuber and rubber band, with each turn touching the previous one, until the edge of the second beaker was reached. This point on the cable was noted and the length required to cover the area of tuber between beakers was measured. The area under the beakers was calculated on the assumption that the protuberances were conical.

The method was tested by repeated measurements of a wooden cylinder with coned ends, the surface area of which could be calculated accurately. The errors of measurement were of the order of 1%. For small samples, therefore, the method was considered to be an improvement on approximations derived from weight and dimensions of tubers.

Preparation of mixtures of air, water vapour and nonyl alcohol vapour

Air saturated with water vapour or with nonyl alcohol vapour was generated by passing dry air through a coiled copper tube and then through a sintered glass gas-washing bottle containing water or nonyl alcohol, the tube and the bottle being immersed in a constant-temperature water bath.

* Part I: *J. Sci. Fd Agric.*, 1966, **17**, 428

Alcohol-air mixtures were allowed to come in contact only with glass or polytetrafluoroethylene (PTFE) surfaces, and these were kept as compact as possible to limit the time taken for the vapour to reach equilibrium with its containing surfaces.[1] Tube joints were overlapped before sealing with rubber tubing. Three arrangements of apparatus were needed for the three types of investigation.

Saturation concentration of nonyl alcohol vapour in dry air

Initially the alcohol vapour generator was cooled to about 0° by addition of ice to the water bath. The bath temperature was then allowed to rise slowly. The effluent alcohol vapour–air mixture was passed (at a rate < 5 l/h) through a short PTFE 'receiving' tube immersed in a second water bath held at a temperature below ambient by a copper tube cooled with refrigerated glycol. In this arrangement the alcohol vapour–air mixture was not diluted with air after leaving the generator.

As in the other two arrangements described below, the float flowmeter was placed on the high-pressure side of the generator. Under the most unfavourable conditions, namely at the maximum flow rate of 5 l/h, the pressure at that point was less than 2% above atmospheric pressure. The consequent error in flow rate determination was comparable with the error involved in reading the flowmeter itself

Saturation concentration of nonyl alcohol vapour in moist air

Air was saturated with water vapour in a bath of melting ice and diluted with dry air previously cooled to 0° by being passed through a coiled copper tube. The mixture was then used to dilute an alcohol–air mixture produced in a second constant temperature bath, and the final mixture was passed through a PTFE 'receiving' tube in the ice bath. It should be noted that the air streams saturated with water or alcohol vapour were diluted at a point before they left their respective baths in order to avoid premature condensation of liquid in the delivery tubes.

Interactions between tubers and mixtures of air, nonyl alcohol vapour and water vapour

This assembly was the most elaborate of the three, and is shown diagrammatically in Fig. 1. The nonyl alcohol vapour generator was immersed in one constant temperature water bath and the water vapour generator and potato treatment chamber in the other. Each bath could be used at temperatures above or below ambient. The treatment chamber was supported on a simple metal cradle to enable it to be withdrawn from the bath with ease. The two glass cocks used to bypass the treatment chamber had glass-filled PTFE keys (G. Springham & Co. Ltd.) and were ungreased. The air streams saturated with vapour were diluted before leaving their respective baths. The tubes carrying these air streams were shielded by passing through a wide glass tube. The standard joints on the treatment chamber were greased in a band at the outer end of the joint to prevent ingress of water.

The apparatus was used in the following way. The required concentrations of water and nonyl alcohol vapour were established with the needle valves A, B and C, the total flow rate of air was adjusted to 5·55 l/h and the flow rate of stream C (nonyl alcohol vapour in air) kept at 0·55 l/h. The treatment chamber was bypassed by means of the

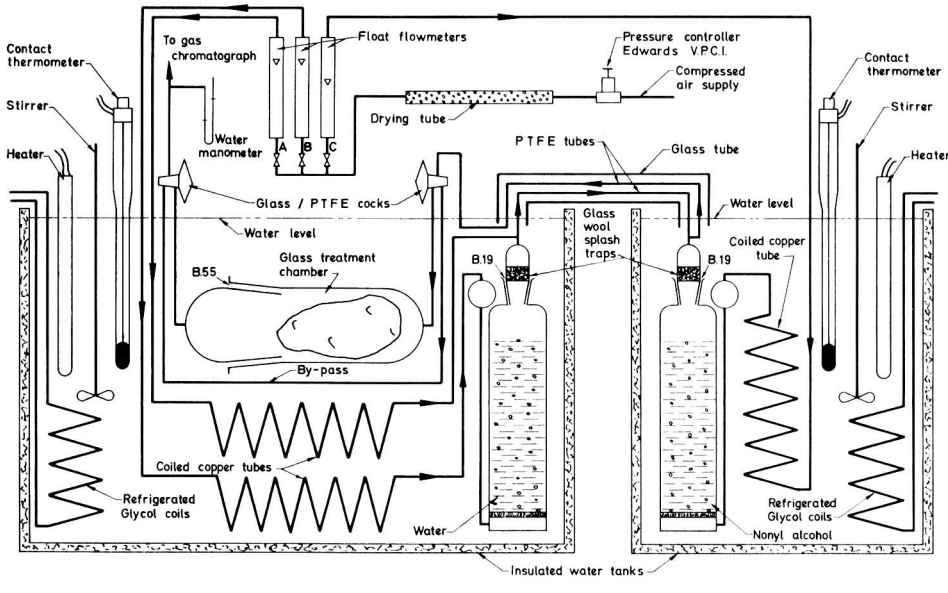

FIG. 1. *Apparatus used to study interactions between tubers and mixtures of air, nonyl alcohol vapour and water vapour*

glass/PTFE cocks, raised from the water, opened and cleansed of alcohol residues for 30 min with a jet of air. The chosen pair of tubers, already at about the desired temperature for treatment, were placed in the chamber and the apparatus was reassembled. After a 30 min equilibration period in the water, and immediately after the chromatograph had taken a sample, the flow of air was directed through the chamber. Some hours later, when sorption of alcohol vapour by the tubers had ceased, as shown by the monitoring gas chromatograph, the humidity of the air was altered, immediately after air sampling, by adjustment of streams A and B (dry air and moist air). After a further hour the alcohol concentration had reached its original level and the run was ended. A blank run without tubers was conducted in the same way.

Measurement of nonyl alcohol vapour concentration

Nonyl alcohol was estimated with a gas chromatograph, fitted with an automatic gas sampler, which took ~0·5 ml samples at 7·5 min intervals. The chromatograph and the method of calibration have been described.[1]

Results and Discussion

Saturation concentration of nonyl alcohol vapour in dry air

Fig. 2 shows a typical result obtained with dry air. Nonyl alcohol vapour concentration rose steadily with the alcohol generator temperature until the latter exceeded that of the receiver by ~0·25°. At this point nonyl alcohol began to condense in the receiving tube and an inflexion occurred in the vapour concentration curve. The slight delay in response can be attributed to the time taken for the vapour generator and air stream to reach the temperature of the water bath. It is worth noting that a more volatile impurity in the nonyl alcohol was detected by the chromatograph, and this, as would be expected, showed no inflexion in the concentration curve (Fig. 2). When the vapour mixture was replaced by air the alcohol concentration dropped rapidly to zero, showing that the amount of alcohol absorbed on the surface of the receiving tube was insignificant. A tube of much greater surface area did not display a sharp inflexion point in this type of experiment.

By repetition of the experiment at different receiver temperatures a number of 'dew points' for nonyl alcohol vapour were determined. The curve in Fig. 3a shows the variation of saturation concentration of alcohol in dry air with temperature. As a test of the reliability of the results, vapour pressure data were derived from the curve by application of the ideal gas laws and Dalton's law of partial pressures. The vapour pressures thus obtained were tested by application of the approximate form of the Clapeyron–Clausius equation:

$$\frac{\mathrm{d} \ln p}{\mathrm{d}T} = \frac{L_e}{RT^2}$$

Where L_e is the molar latent heat of evaporation.
If it is assumed that the latent heat L_e is constant over a small range of temperature then:

$$\log p = c - \frac{a}{T}$$

Where a and c are constants for the given substance.
When the logarithms of the vapour pressures were plotted against the reciprocals of the absolute temperatures the straight line expected from the above equation was obtained (Fig. 3b). Calculation of the molar latent heat of evaporation

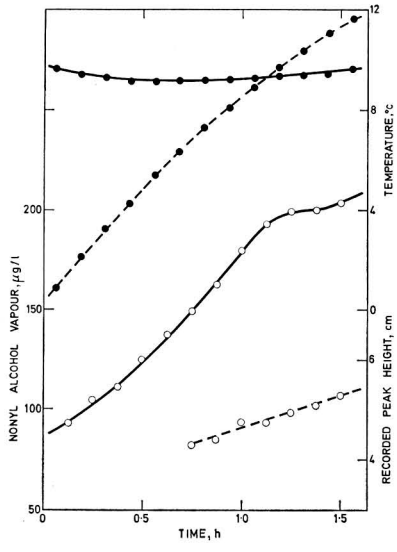

FIG. 2. *Experimental determination of the saturation concentration of nonyl alcohol vapour in air*

Temperature of alcohol generator
Temperature of receiver
Concentration of alcohol vapour
Concentration of more volatile impurity

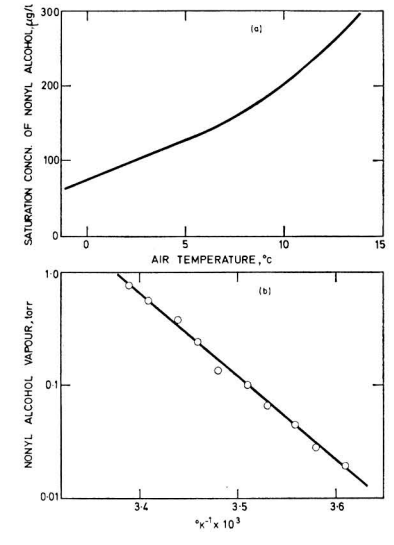

FIG. 3 (a). *Variation with temperature of the saturation concentration of nonyl alcohol in dry air*

(b). *Variation with temperature of the vapour pressure of nonyl alcohol, calculated from saturation concentration data*

of nonyl alcohol from this curve gave a value of 14,500 cal/mole.

Saturation concentration of nonyl alcohol vapour in moist air

Since under normal conditions water vapour is an unavoidable component in the air–nonyl alcohol–potato system it was necessary to establish whether the presence of water vapour changed the saturation concentration of the alcohol in air. The results of a number of experiments carried out at constant flow rate and a receiver temperature of 0° in the presence of varying concentrations of water vapour are shown in Fig. 4. These exhibit a disappointing irregularity, thought to be due to the use of a relatively long receiver tube in which adsorption effects would be accentuated. It can be seen, however, that at 0° a change in % R.H. from 0 to 90 would cause a depression of only about 7 μg/l in the saturation concentration of nonyl alcohol. At higher temperatures the depression might be greater.

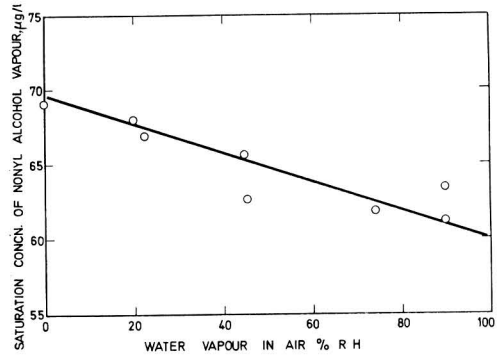

FIG. 4. *Variation with relative humidity of the saturation concentration of nonyl alcohol in air at 0°*

Interactions between tubers and mixtures of air, nonyl alcohol vapour and water vapour

Preliminary experiments

These gave a general picture of the way in which nonyl alcohol was taken up by the tuber and of the effect of changes in atmospheric humidity on a tuber already in equilibrium with a mixture of air and alcohol vapour.

The results of one of the sorption experiments are shown in Fig. 5. The graph compares the concentration of alcohol vapour in the air entering the treatment chamber with the concentration in the effluent air. The curve for the latter fits an exponential equation of the form

$$C_t = C_0 \exp\left(-\frac{t}{T}\right)$$

where C_0 is the difference between the applied and the effluent alcohol vapour concentration produced by the tuber at time 0, C_t is the corresponding difference at time t, and T is the exponential time constant measured in hours (i.e. the time taken for the rate of uptake to decrease to 1/exp x, where x is the original value).

In practice the values of C_0 and C_t were obtained from a logarithmic straightline plot and the value of T was calculated

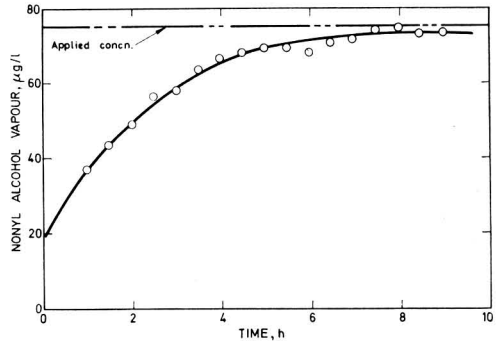

FIG. 5. *Uptake of nonyl alcohol by potato tubers exposed to a mixture of dry air and nonyl alcohol vapour at 5·5°*

—— ·· —— ·· —— applied alcohol vapour concentration
———————— concentration of vapour in effluent

from them. No points are shown for the first hour of the experiment because blank runs indicated that during the first hour nonyl alcohol was being adsorbed by the walls of the chamber and clearing the chamber of pure air. This produced slight deviations from the theoretical curve; these could be ignored because they tended to cancel each other out. The total mass of alcohol taken up by the tuber was calculated from the area A between the two curves given by the integrated form of the exponential equation, $A = C_0T$.

The effect of changes of humidity on a tuber treated with nonyl alcohol are shown in Fig. 6. In the experiment shown in Fig. 6a a tuber was being treated with dry air containing alcohol vapour (0·16 μg/l). At time 0 the chamber was bypassed for about 30 min before ventilation of the tuber was resumed. After a brief drop the alcohol vapour concentration in the effluent air rose steeply to a prominent peak and then fell to a level below that of the entering air before slowly regaining the original level. Thus when the chamber was closed nonyl alcohol was released by the tuber. When this experiment was repeated with a vapour mixture of high humidity there was no release of alcohol. In the experiment shown in Fig. 6b a tuber at 22° was in equilibrium with a dry mixture of air and nonyl alcohol vapour. The temperature of the chamber was gradually raised to 31° and then held constant. As a result the tuber released a little alcohol as shown by the rise in the curve. At about 1·5 h the relative humidity of the vapour stream was raised from 0 to about 75%. A comparatively large quantity of the alcohol was then released during about 30 min. This confirmed that if a slight rise in the temperature of the tuber occurred, owing to accumulation of metabolic heat or ambient changes, it would not cause so pronounced a release of alcohol as a substantial change in atmospheric humidity. In the experiment shown in Fig. 6c a tuber in equilibrium with a moist mixture of air and nonyl alcohol vapour was treated at time 0 with a dry mixture of the same alcohol content. After a time lag of 1·5 h the tuber took up alcohol more rapidly for about 1 h. The shape of the curve suggested that a drying process was taking place on the surface of the tuber.

Quantitative study

In a quantitative study of the effect of temperature, humidity

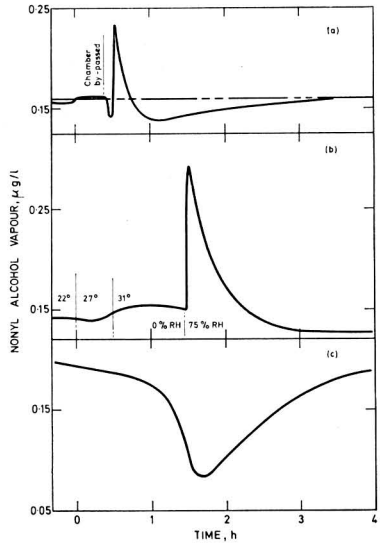

FIG. 6. *Effect of changes in atmospheric humidity on potato tubers exposed to nonyl alcohol vapour*

(a) ventilation stopped at time 0 and resumed at about 0·5 h
—— ·· —— ·· —— applied alcohol vapour concentration
———— concentration of vapour in effluent.
(b) addition of water vapour (to about 75% R.H.) from 1·5 h onwards
(c) substitution of dry air for moist air at time 0.

and applied nonyl alcohol concentration on the tuber, independent measurements were made of the rate and extent of alcohol uptake and the amount of alcohol transferred to or from the tubers after a change in humidity. Pairs of potatoes were treated with mixtures of air, alcohol vapour and water vapour at two temperatures (5·5° and 11·4°), two humidity levels (0% and 80% R.H.) and three alcohol vapour levels (~35, 80 and 160 µg/l). The order of the treatments was randomised to reduce the risk of possible changes in the tuber samples during the period of the experiment.

The 80 µg/l level was close to the recommended commercial application rate of 100 µg/l.[2,3] The three concentrations were chosen to provide two 2 × 2 × 2 factorial experiments. In one, the concentration of alcohol was considered in absolute terms (35 and 80 µg/l) but in the other the concentration was considered in terms of % saturation. Here the 80 µg/l treatment at 5·5° and the 160 µg/l treatment at 11·4° provided nonyl alcohol vapour at about 70% saturation while the 35 µg/l treatment at 5·5° and the 80 µg/l treatment at 11·5° provided alcohol vapour at about 30% saturation.

The results of these experiments in terms of equilibration rate (using the exponential time constant), weight of alcohol taken up by the tubers and weight of alcohol taken up or released after a change in humidity are shown in Table I. A statistical analysis was made of the factorial experiment in which the concentration of alcohol vapour was measured in terms of % saturation. This yielded more consistent results than the one in which absolute units were used. Of the three variates measured (time constant, total uptake of alcohol and change of alcohol content caused by a change in humidity), the first two were submitted to an analysis using the single third-order interaction variance to assess error. However, when no interactions were observable the three second-order and one third-order interaction variances were pooled in order to compare the main effects with more precision. The new error variance was then based on four degrees of freedom for each variate. The results are shown in Table II. They indicate that a rise in temperature tended

TABLE I

The effects of temperature, humidity and nonyl alcohol vapour concentration on the rate and extent of uptake of alcohol by tubers, and of a change in humidity on the alcohol content of tubers

Temperature °C	Humidity % R.H.	Nonyl alcohol vapour concentration		Tuber Weight g	Uptake of nonyl alcohol			
					Equilibration rate, exponential time constant, hours		Total uptake, µg	Change in content after a change in humidity** µg
		% saturation	µg/l air		Found	Corrected*		
5·5	0	26	35	125	3·84	3·86	598	−192
5·5	0	57	76	123	2·31	2·31	781	0
5·5	80	23	30	124	4·67	4·64	266	0
5·5	80	52	69	125	3·78	3·85	695	+ 68
11·4	0	16	35	123	2·12	1·99	92	− 80
11·4	0	37	84	125	1·65	1·68	297	−168
11·4	0	69	158	123	1·75	1·81	1170	− 31
11·4	80	13	30	127	0·78	0·73	60	+ 66
11·4	80	33	75	124	2·18	2·21	182	+165
11·4	80	74	171	124	2·74	2·66	757	+ 40

* The exponential time constant was corrected for the surface area difference between samples, using the mean surface area of all pairs of tubers as the base. The second sample was of mean surface area

** The change in humidity was made after equilibration with the nonyl alcohol vapour. Samples treated in air of 0% R.H. were changed to air of 80% R.H. or the reverse

\+ indicates a gain of alcohol by tubers

− indicates a loss

TABLE II

Statistical analysis of the effect of temperature, humidity and concentration of nonyl alcohol on the rate and extent of uptake of alcohol by potato tubers

	Equilibrium time constant		Total uptake of alcohol	
	Variance ratio test, % significance	Difference between means, hours†	Variance ratio test, % significance	Difference between means, μg†
Temperature, 5·5 and 11·4°	2·5	−1·58 ± 0·77*	—	—
Applied humidity 0% and 80% R.H.	10	+0·93 ± 0·43*	20	−237 ± 161
Applied alcohol concentration <30% and 70% saturation	—	—	2·5	+490 ± 328

† The positive or negative sign before the figure indicates a positive or negative correlation of the variate with the factor. Figures preceded by the ± sign are standard errors
* A statistical 't' test gave a significant result at the 5% level of probability

to reduce the equilibration time, perhaps because the rate of dissolution and diffusion of nonyl alcohol in the potato increased. A rise in atmospheric humidity increased the equilibration time and decreased the extent of alcohol absorption.

The nature of the variate, change of alcohol content caused by a change in humidity, rendered it unsuitable for simple statistical analysis. In Fig. 7a are shown the effects of applied alcohol concentration and humidity on total uptake of alcohol at 11·4°. The amount of alcohol taken up by the tuber increased as the square of the vapour concentration, the relationship for dry air being $m = 0{\cdot}0485\ c^2$ and for air at 80% R.H. $m = 0{\cdot}028\ c^2$ (where m μg of alcohol are taken up by the tuber at an applied alcohol concentration of c μg/l). Thus less alcohol was sorbed by the tubers in moist air. In Fig. 7b the corresponding effects of applied alcohol concentration on alcohol uptake or release after a change of humidity are shown. After this change, tubers in a moderate concentration of alcohol experienced the greatest gain or loss of alcohol.

Conclusions

The experiments, in which sudden humidity changes were made, indicated (Fig. 6) that part of the nonyl alcohol associated with the tuber can be removed or replaced in a short time. On the other hand, when a tuber is first exposed to alcohol vapour or when a tuber containing alcohol vapour is exposed to pure air it takes many hours to establish equilibrium. Since the alcohol has a relatively low volatility this suggests that the alcohol present at the surface of the tuber is adsorbed as a fairly concentrated film by forces of the van der Waals type; equilibrium is rapidly established there. The remainder of the alcohol is held in solution or otherwise sorbed in the body of the tuber, where the process of diffusion away from the surface is slow.

Atmospheric humidity has some effect on the total alcohol content of the tuber (Fig. 7a) and a marked effect on the surface film of alcohol. It appears that water molecules compete with alcohol molecules for adsorption sites on the skin. When moderate alcohol concentrations were present in the air, changes of humidity from moist to dry air, or the

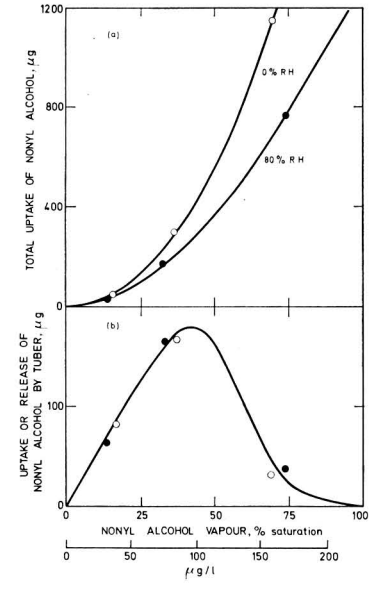

FIG. 7. *Effect of atmospheric humidity and applied nonyl alcohol vapour concentration on the uptake or release of alcohol by potato tubers at* 11·4°

(a) total uptake under constant humidity conditions
○ 0% R.H.
● 80% R.H.
(b) change in nonyl alcohol content resulting from a change from 0 to 80% R.H. (release) or from 80 to 0% R.H. (uptake)
● uptake
○ release

reverse, caused a relatively large amount of alcohol to be adsorbed or desorbed respectively (Fig. 7b). At low application rates the smaller effect of a humidity change can be attributed to the relative scarcity of alcohol molecules on the skin surface. At high application rates the surface equilibrium appears to move against water in favour of the nonyl alcohol.

The determination of the saturation concentration of nonyl alcohol at various temperatures has provided data which can be used to predict the conditions in which condensation of alcohol is likely to occur on stored tubers and which from past experience is likely to cause severe damage.

The results of the tuber experiments have certain implications for commercial practice, though to implement them might well be impracticable for economic or other reasons. To transfer a large amount of nonyl alcohol rapidly to a tuber it is found to be necessary to apply the dry vapour at high concentration and keep the atmosphere dry and cool thereafter. However if a high concentration of alcohol is required only in the atmosphere, the vapour should be applied in dry air until equilibrium is reached, and the humidity then allowed to rise. It is not known which of these two methods will produce the most effective sprout suppression; a comparison might be of value.

Acknowledgments

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References

1. Meigh, D. F., & Currah, I. E., *J. Sci. Fd Agric.*, 1966, **17**, 428
2. Burton, W. G., *Eur. Potato J.*, 1958, **1**, 42
3. Burton, W. A., *Agriculture, Lond.*, 1958, **65**, 299

STUDIES ON THE NORTH SEA COD
VI.—Effects of starvation 4. Sodium and potassium

By R. M. LOVE, I. ROBERTSON and I. STRACHAN*

Sodium and potassium were measured in captured cod of a variety of degrees of starvation, and in cod artificially starved in an aquarium. It was found that depletion of either kind caused the ash of both muscle and blood to decrease, while the water increased. In muscle, Na increased and K decreased, but both ions decreased in the blood plasma. It was demonstrated histologically that conditions producing more Na and less K gave rise to an increase in extracellular space and shrinkage of the muscle cells.

Myocommata (sheets of connective tissue) were found to be rich in Na. As the myocommata are more concentrated in the tail region, this alone was thought sufficient to explain the high Na concentration in the flesh at the tail ends of many fishes compared with the middle.

A clear relationship was demonstrated between the size of the fish and the Na content of the musculature. This decreased with growth, a fact which was thought to be the result of larger fish having larger cells, and therefore less extracellular space which is rich in this ion. It was found that there was no relationship if the fish were depleted in the spawning season.

There was in addition an unknown factor whereby both Na and K showed an overall shift from month to month.

Introduction

In previous papers of this series the influence of anatomical locality[1,2] and starvation[3–5] on the chemistry of the muscle tissue of cod (*Gadus morhua*) have been considered. By studying the dynamics of chemical composition it is hoped to acquire a deeper insight into the physiology of depletion, and incidentally to see more fully the rôles of the various constituents in the living material.

Other work[6] showed that the water content of the muscle was a reliable indication of the condition of the fish, increasing whenever the fish starved. In the present paper, therefore, the Na, K and total ash are examined with especial reference to the water content, not merely to the period of starvation.

Experimental

Unless stated otherwise, all the experiments were carried out with cod which had been caught by trawling in the vicinity of Aberdeen. 'Dead' fish for seasonal surveys were kept, without gutting or washing in sea-water, in dry boxes at an ambient temperature near 0°, and were gutted, filleted and

* Present address: College of Fisheries, St. John's, Newfoundland

sampled within a few hours of death. This procedure was adopted to prevent the leaching of inorganic constituents, demonstrated in the following experiment.

5 fish, each weighing about 3 kg after gutting, were packed into ice made from de-ionised water. This was contained in a stainless steel box fitted with a perforated stainless steel base and funnel for collection. The whole was placed in a room at 3°, and the melt-water from the ice was collected at intervals, evaporated and ashed at 500°. A total of 1 to 1·5 g ash was found to be leached from the fish every 24 h, and its composition varied as shown in Fig. 1. While it might be assumed that the Na-rich and K-rich tissues were sufficiently intertwined to cause rapid mingling of the ions at death, this experiment shows that equilibrium is not reached until many days later, at least at the surface of the fish. Little K from the interior of the cells reached the surface for several days, so that at first the melt-water contained mostly Na ions, presumably from the skin. There is, however, a fairly rapid decline in the proportion of Na as that from the skin and extracellular fluid near the surface of the fish is washed away, and a cross-over point occurs about 7 days after death, after which the K, the major ion of the body, predominates. It was therefore considered vital to sample the muscle as soon after death as possible to obtain meaningful results.

Live fish for the starvation experiments were kept in aquaria maintained at 9°. Controls were fed three times per week with as much chopped muscle of squid (*Loligo forbesii*) as they would eat.

Samples, free from skin or bones, were dried in silica crucibles at 100° for 1 week for the determination of water by weight loss, then ashed in an electric furnace at 500°. The ash was dissolved in a little mineral-free hydrochloric acid and diluted with de-ionised water. All solutions and standards were kept in bottles made of polyethylene.

Na and K were determined by emission photometry, using a Hilger 'Uvispek' with flame attachment.

Histological specimens of muscle from starving fish were prepared immediately after death by cutting out a 5 mm cube of muscle from the surface of the fillet next to the bone. These samples were left in 4% formaldehyde for 2 days or more and then dehydrated in dioxan, embedded in paraffin and finally sectioned at 5μ. The sections were stained with van Gieson's picrofuchsin.

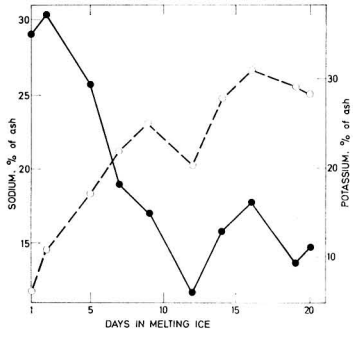

FIG. 1. *Composition with time of the ash from the melt-water from 5 gutted cod packed in de-ionised ice at 3° ambient. The fish were captured on day* 0

●——● Na ○- - -○ K

Results and Discussion

Anatomical distribution

It has been reported that the concentration of sodium increased from head to tail in *Hippoglossus hippoglossus*,[7] *Oncorhynchus gorbuscha*[8,9] and *Salvelinus namaycush siscowet*.[10] Kruchakova[11] reported the same relationship in a number of species of fish from the Black Sea, and suggested that the tail muscle had a higher Na content because it contracted more actively than the main body muscle during swimming, noting that the jaw muscles of predatory fish which were even more actively contracting, had the highest concentration of all. Kruchakova also reported a similar increase in K, but as this was not found in the species examined by Thurston,[7-9] or in the present work, it is evidently not a general phenomenon.

However, from studies on carbohydrates and their metabolites, Black *et al.*[12] concluded that there was *no* indication that the tail muscle was more active than the main body muscle, and that there was almost always a lower concentration of glycogen, the chief energy precursor for aerobic metabolism, in the tail than in the middle section of the fish musculature.[13-15] Furthermore, carbohydrate intermediates such as sugar phosphates are not preferentially concentrated in the tail region.[15] The breakdown of lipid for energy purposes may be less in the tail than elsewhere, since the lipase activity of ling cod, *Ophiodon elongatus*, has been shown to decrease steadily towards the tail,[16] although this result may not be typical of other species, since the shape of ling cod is unusual. Thus it is by no means certain that Kruchakova's explanation[11] for the increased concentration of Na in the tail muscle is the correct one.

In the present work the myotomes (muscle tissue) and myocommata (sheets of connective tissue joining the myotomes) were separated by dissection[17] before analysis. The results are shown in Table I.

TABLE I

Concentrations of Na and K in muscle from the head and tail ends of cod fillets
(mg per 100 g fresh weight)

	Na	K
Whole flesh from head end	36·9	472
Whole flesh from tail end	66·9	396
Myotomes freed from myocommata, head end	23·7	442
Myotomes freed from myocommata, tail end	33·9	424

These figures were obtained from a cod 65 cm long from the aquarium; it was killed on 7 October 1960 and filleted and dissected immediately. Since myocommata have a big surface/volume ratio, it was considered essential to use only freshly killed material; appreciable diffusion of ions in various mammalian organs soon after death has been demonstrated by several workers.[18-22]

Dissection of the anterior myotomes posed no problems, the scalpel being used with a sawing action, but the myotomes from the tail end were very narrow and interspersed with connective tissue, so that this method could not be used. Instead, the surface of the tail end of the fillet was repeatedly scraped with a scalpel blade, on which muscle tissue gradually accumulated leaving glistening strands of connective tissue behind. There is a possibility that the separation of the two

types of tissue at the tail end by this means was not complete.

The figures in Table I confirm that the concentration of Na in flesh increases from head to tail in cod, and that the K decreases. Dissection of myotomes free from myocommata reduced the concentration of Na to 64% of the value in the whole flesh at the head end and to 51% at the tail end, showing that myocommata are relatively rich in Na, and that therefore an increase in the proportion of myocommata, as found in the tail region, could account for the increase in Na in whole flesh.

However, Na increased towards the tail in the dissected myotomes also, although here it was not as great an increase (43%) as in the whole flesh (81%). If the increase towards the tail in whole flesh were caused solely by an increase in the proportion of myocommata, there should have been no increase at all after dissection. There are two possible reasons for the increase in the dissected myotomes at the tail end. Firstly, the mechanical separation may not have been complete, and secondly it was shown in the first paper of this series[1] that the diameters of cells at the tail end of cod were much narrower than at the head end. The film of fluid surrounding each muscle cell is rich in Na, as has been demonstrated directly in cod by separation of the pure cell contents.[23] If a unit volume of tail muscle, compared with muscle from the head end, contains a greater number of smaller-diameter cells, it is to be expected that the proportion of extracellular fluid, and that of Na, will increase also. Both of these factors may account for the figures for dissected muscle shown in Table I, but their relative contributions are not known.

In the next experiment, the Na and K contents of myocommata themselves were measured. The isolation of myocommata by dissection can be difficult, so small fish having thin myocommata are not suitable. The experiment was therefore carried out at sea (off the West coast of Scotland) in June 1959 so that large fish could be obtained and dissected as quickly as possible. Two cod measuring 84 and 92 cm were dissected and the myocommata were pooled in a single sample. The results (Table II) have been expressed as g per 100 g ash rather than on a fresh weight basis which might have been unreliable under the conditions existing. Eight smaller fish (about 75 cm) yielded between them a further single sample which was sufficient for analysis, and the results are also quoted in Table II.

TABLE II

Concentrations of Na and K in myotomes and myocommata of cod (g per 100 g ash)

	Na	K
Myotomes from 2 large cod	7·1	24·4
Myocommata from the same cod	18·8	18·9
Myotomes from 8 smaller cod	4·0	30·8
Myocommata from the same cod	19·3	22·0

There is fair agreement in the two sets of fish, showing that myocommata possess considerably more Na than myotomes, and less K. Muscle cells possess a mechanism for extruding Na, but no such mechanism exists in the connective tissue. In general, the levels of electrolytes in connective tissue per litre of water closely resemble those in a plasma ultra-filtrate;[24] indeed, it has been suggested that connective tissue acts as a store for the deposition or mobilisation of Na and chloride.[25]

Fish size

A relationship between the size of an animal and the mineral composition of the musculature has never been satisfactorily demonstrated. In rats, the K and Ca of the muscle gradually diminishes between the ages of 1 and 36 months.[26] In man,[27] pig,[27] and poultry[28] on the other hand, K increases and Na decreases during growth. In fish, the picture is also confused. *Sebastodes alutus*, *Gadus macrocephalus* and *Ophiodon elongatus* have been shown[29] to be quite uniform in mineral composition, regardless of species, size, season or fishing ground. However, the Na and K contents of *Anoplopoma fimbria* vary considerably.[29] In an extensive table, McBride & MacLeod[30] showed that the Na content of *Oncorhynchus tschawytscha* muscle was greater in three small fish out of five, compared with medium or large fish, but in *Hippoglossus stenolepsis* the larger fish showed the higher values. Later, it was confirmed[31] that the Na and K levels varied with size, but the issue was complicated by the fish spending part of their time in salt water and part in fresh, and the authors felt that a number of variations in the Na and K levels could not be explained.

It was realised in the present work that any investigation would need to take in most seasons of the year as well as accounting for size. Note was also made of the nutritional status of the fish as measured by myotome water content, values of 80·9% or below being regarded as normal, and 81% or above as relating to starving fish.[6] The sizes were restricted to some extent by the season—very large cod are found near Aberdeen only in the winter time—so the size groupings are not exactly the same for each month. Where muscle samples from several fish were grouped together, equal weights of material were taken from the anterior half of each fillet, dissected free from connective tissue, and mixed together after ashing. The figures for male and female fish have been combined, since no consistent differences appeared between them.

The results in Table III show that *in all months where the water content of the fish was less than* 81% there was a clear inverse relationship between size and Na content. K is not so related, and the concentration appears to vary randomly within each month, and in addition there seems to be a change in overall level between months. There is also a noticeable month-to-month variation in the Na content of the smallest size-group, which is seen even when the water content has not changed. Its significance is unknown.

In March and April, when the water content of the musculature rose, the relationship between Na and fish size was obscured. As will be shown later in this report, depletion causes muscle Na to rise, and as bigger cod become more depleted at the spawning time than smaller cod,[6] they exhibit an augmented rise in Na which more than cancels out the relationship caused by size alone. As an example, the largest fish caught in April (Table III) have the highest Na concentration of all the size-groups of that month, although in the 'non-starving' months they invariably have the lowest.

Depletion

The content of total ash in cod muscle has been shown to decrease during the build-up of the gonads, with a concurrent decrease in protein and increase in water.[32] When cod were artificially starved to the point of death,[3] the ash in the muscle fell from about 1·2 to below 1%, the value for the most depleted fish being as low as 0·797%. The reduction is probably related to the depletion of protein from the

TABLE III

Concentrations of Na and K in myotomes of cod of different sizes at different times of the year, in relation to nutritional condition
(mg per 100 g fresh tissue)

Date caught	Number of fish	Size range (cm)	Water content %	Na	K
4 November 1958	10	51–55	80·5	43·7	687
	14	64–79	80·8	47·2	593
	3	81–86	80·4	43·6	658
	1	95	80·3	37·3	639
	1	106	80	37·1	863
21 January 1959	10	51–63	80·4	28·6	470
	9	64–75	80·4	30·4	370
	8	76–99	80·4	26·2	439
17 March 1959	7	51–64	81·5*	27·9	594
	10	65–76	82*	33·0	637
	4	84–109	83·5*	47·2	594
	1	86	80·4	30·3	652
	2	86, 99	83·1*	38	629
23 April 1959	20	38–64	80·7	30·1	545
	10	65–75	81·7*	33·2	595
	4	76–101	82·7*	31·6	538
	2	82, 92	82·8*	47·8	526
30 June 1959	20	25–51	80·6	41·5	528
	10	69–81	80·5	34·8	514
29 July 1959	19	25–51	80	47·2	422
	9	69–81	80·3	46·6	446
	1	89	80·2	37·6	415
14 October 1959	20	under 64	80·7	60·1	419
	14	64–89	80·4	42·2	398
	1	90	79·6	33·6	425

* Fish undergoing depletion

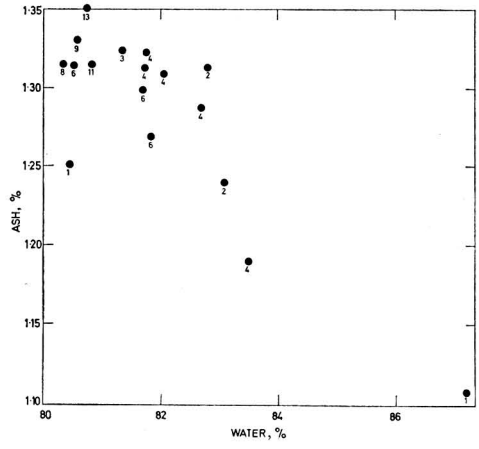

FIG. 2. *Relationship between ash and water in the muscle of cod caught in the North Sea.* Small figures show the number of fish represented by each point.

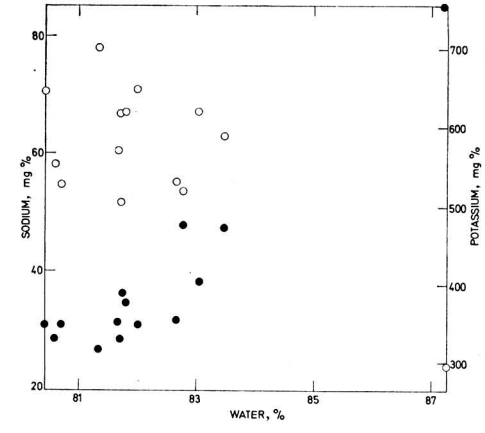

FIG. 3. *Sodium and potassium in the muscle of cod of different water contents*
● Na ○ K

muscle, because among fatty fish, which use fat instead of protein in times of dearth, herring do not show seasonal variation in the ash content,[33] neither do salmon show any change during spawning migration.[34]

The relationship between ash and water content in the muscle is shown in Fig. 2, the fish being the naturally-starving cod caught in March and April and the replete cod caught in October 1959, values for males and females being shown separately. The figures for the other months were found to be grouped near 1·3%, since the water content never rose above 80·9%. The extreme value at 87% water relates to a single fish caught off the West coast of Scotland on 10 June 1959. It will be seen that the decline in ash becomes serious only for values of water content above 83%.

Fig. 3 shows the corresponding figures for Na and K for March and April together with the single value for June. Again, it is only above about 83% water that the Na and K begin to show changes; this agrees with the observations of Sutton.[35]

Although it is clear that a number of substances, such as the different solubility fractions of protein,[4,17] vary seasonally and can be correlated with changes in the water content, this does not establish that simple depletion is necessarily the cause. The growth of the gonads causes gross anatomical changes at the same time, and the hormone pattern of the blood changes profoundly: it has in fact already been tentatively suggested[31] that hormones might be involved in changes in the mineral constituents of salmon. For the next experiment, therefore, some cod were subjected to starvation in an aquarium at a time when the gonads were inactive.

The fish, which were in the size range 50 to 70 cm were caught on 18 July 1960 and placed into clean water three days after capture. After starvation for 4, 8 and 12 weeks, 6 fish were removed from the aquarium, stunned, filleted and sampled, myotomes from the anterior half of the fillet being dissected free from myocommata. At the end of this time, the remaining fish were fed on as much squid muscle as they would eat, and after 14, 16 and 18 weeks, 5 fish were removed for sampling. After 22 weeks (10 weeks of feeding) 3 fish

were sampled, the smaller number being the result of some deaths in the aquarium. The amounts of water and ash in the muscle (Fig. 4) change according to previous experience: water increases and ash decreases during depletion with the reverse occurring during re-feeding. Fig. 5 shows the corresponding figures for the blood, which are seen to follow a trend similar to that of muscle. Fig. 6 answers the main question, showing that starvation alone, without the intervention of hormones, is able to cause an increase in the Na and decrease in the K of muscle. It will be noticed that although the water content in the muscle and possibly also the ash (Fig. 4) shows an immediate response to feeding at the end of the starvation period, there is a delay in the recovery of Na and K, both of which continue their starvation trend for a further 2 weeks or so. It was shown earlier[4] that the total protein N of the muscle behaved in the same way. Fig. 7 shows the changes of Na and K in the blood. Here there is a general decrease in *both* ions during starvation and an increase during re-feeding, in contrast to work by Sutton[35] who observed little change.

The results in Fig. 6 agree well with a number of published observations. In *Oncorhynchus nerka*, the Na has been shown to increase during spawning migration,[36] and the jellied condition, a characteristic of severe depletion,[37] has been shown to result in increased Na in the muscle of *Microstomus pacificus*.[38] Inverse relationships have also been reported between Na and K. In the goldfish, *Carassius auratus*, it was calculated that for every milliequivalent of K lost, 1·2 milliequivalents of Na were gained,[39] and Thurston[8] has concluded that a high level of Na was associated with a low level of ash, shown in the present work to indicate depletion. He also concluded that a high level of K was associated with a high level of protein, but in another publication with co-authors[40] there seemed to be little correlation between Na and K, or between either of them and water, fat, ash or protein. If the relationship is as clear as the present work suggests, it is evidently restricted to certain experimental conditions.

None of these results suggests *why* the Na should increase during depletion, though it was noted in the description of the jellied condition[37] that the extracellular space was enlarged. Since the fluid in the extracellular space is thought to be rich in Na, the starved muscle probably gains Na simply by an

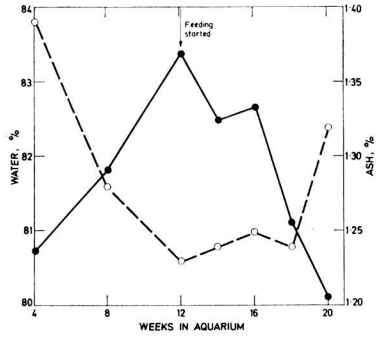

FIG. 4. *Changes in water and ash in the muscle of cod artificially starved in an aquarium at* 9°. Starvation started at 0 weeks, and feeding was commenced at the point shown by the arrow.

●——● Water ○- - -○ Ash

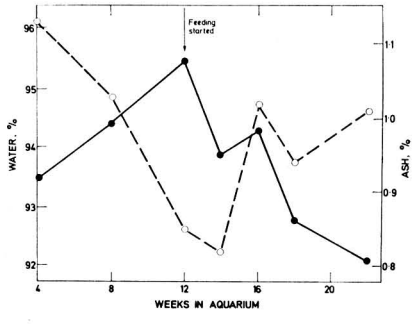

FIG. 5. *Changes in water and ash in the blood plasma of cod artificially starved in an aquarium at* 9°. Feeding commenced at point shown by arrow.

●——● Water ○- - -○ Ash

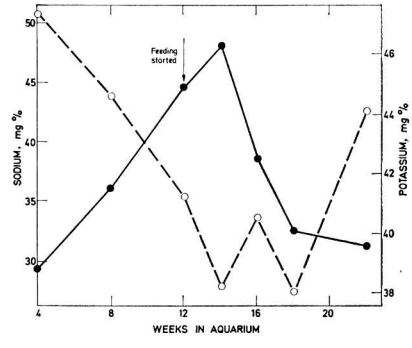

FIG. 6. *Effect of starvation and re-feeding in an aquarium at* 9° *on the sodium and potassium of the muscle of cod.* Feeding commenced at point shown by arrow.

●——● Na ○- - -○ K

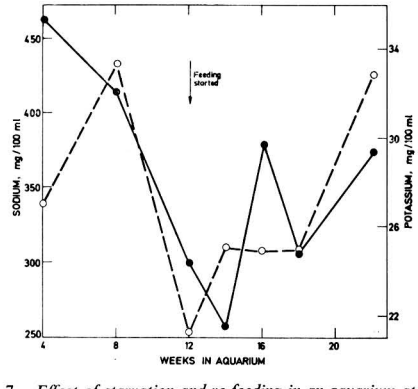

FIG. 7. *Effect of starvation and re-feeding in an aquarium at* 9° *on the sodium and potassium concentration of the blood plasma of cod.* Feeding commenced at point shown by arrow.

●——● Na ○- - -○ K

increase in extracellular space. This was in fact assumed to be the mechanism in Sutton's investigation of starved cod,[35] which showed that Na and chloride increased as the total muscle water increased.

A direct correlation between changes in the Na concentration and the extracellular space itself is, however, difficult to demonstrate, since the proportion of 'extracellular space' differs according to the method of measurement used. 'Sodium space' cannot be used in this instance. 'Chloride space' cannot be used, because the concentrations of Na and chloride are related for many tissues,[41] and also the chloride ion is not exclusively extracellular[42–45] so that extracellular space calculated from chloride concentrations is often larger than by other methods.[46] Since the ion tends to accumulate in fibrous tissue[42] the results may not truly reflect extracellular space (i.e. fluid) in view of the connective tissue strands that run between fish muscle cells (Love & Lavéty, unpublished results). Extracellular space calculated from the diffusion of large molecules such as inulin into pieces of muscle suspended in a bath has not so far been correlated with starvation or increase in water content.

It was reported earlier[3] that extracellular space as measured histologically did not differ significantly between starved and fed fish. However, the fish had already passed through *rigor mortis* before the blocks of muscle were fixed for sectioning. This would cause the extracellular fluid to increase, and might have smoothed out any difference existing initially between the two groups.

In the present experiment, cod caught on 22 June, 1967 (the sizes ranging from 40 to 80 cm) were kept entirely without food for up to 14 weeks, 4 fish being sampled at 0, 1, 2, 3, 5, 7 and 9 weeks, and 3 fish at 11 and 14 weeks. The trends shown in Figs 4 and 6 were confirmed, although the starting levels of Na and K were somewhat different and the effects of depletion were slow to appear.

Blocks of muscle were prepared for sectioning from each fish, and representative pictures are illustrated in Fig. 8.

Extracellular space is still an extremely thin film between the cells after starvation for 1 and 5 weeks and can scarcely be detected in the first two pictures. After 11 weeks, elongated spaces appear, and are distributed all over the picture illustrated (c), and also those made from the muscle of the other 2 fish in the sample. However, this small change is not reflected in the Na or K values, confirming Sutton's observation[35] that little change occurs at water contents below 83%. The muscle of individual fish starved for 14 weeks is illus-

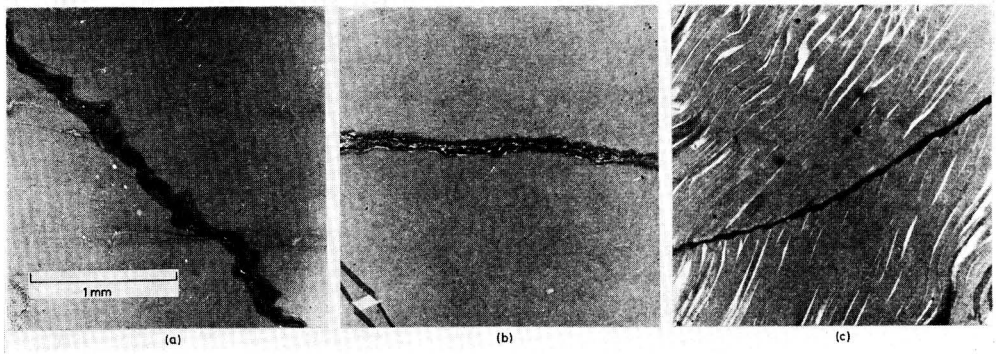

FIG. 8. *Sections of muscle, fixed immediately after death, from cod subjected to starvation for various periods*

Picture no.	Period of starvation (weeks)	Water content %	Sodium mg %	Potassium mg %
a	1	80·3	55·6	514·3
b	5	81·2	60·0	485·6
c	11	82·3	51·6	491·4
d	14	83·3	73·8	438·1

The dark line across each picture is connective tissue.

trated in Fig. 8 (d). The muscle cells are shrunken and the extracellular space is enlarged. At the same time there was an increase in the Na and a decrease in the K level. The water content was 83·3%, signifying a lower rate of depletion than in the experiment illustrated in Fig. 4, where this figure was exceeded in the muscle after only 12 weeks without food. The reserves of the fish in the present experiment must have been greater, since the temperature of the aquarium was the same each time.

Conclusions

It appears that in order to function properly, the contractile muscle cells cannot tolerate large fluctuations in internal water content. When high values are found in whole muscle, they occur as a result of increases in the extracellular fluid, which is said to be rich in Na, and hence the overall Na figure rises. No direct analysis has ever been done on extracellular fluid uncontaminated by sarcoplasm, oedema fluid or blood, because of the problems of isolation. It is assumed, however, that extracellular fluid has the same composition as an ultra-filtrate of blood, and that this accounts for its high Na content.

The lowered K values appear, from Fig. 8, to be the result of the shrinkage of cell material, and the inverse relationship between Na and K observed by other workers[8,39] is therefore consistent.

However, there are some inconsistencies which have yet to be resolved. Figs 4 and 6 show that the changes in Na and K occur smoothly throughout the starvation period, increase in water corresponding to an increase in Na and a decrease in K. On the other hand, Sutton's work[35] showed that Na and K altered appreciably only when the water content had exceeded 83%, and Fig. 3 and the Table in the caption to Fig. 8 show results of the same sort. It is concluded that there are variations in the extent to which extracellular space increases with increasing net water content.

Since, in some cases at least, the water content of whole muscle at the beginning of starvation increases without a corresponding increase in extracellular fluid, then it follows that there is an upset within the cell in the normal ion–water–protein balance. The result is seen in an overall increase in the opacity of the muscle, both in naturally and artificially depleted fish, which has been interpreted as a precipitation of some fractions of the protein owing to this change in balance.[47]

Another inconsistency is seen in Fig. 8, which seems to show a definite increase in extracellular fluid after starvation for 11 weeks, while the analyses still give no change in the Na or K level. A possible reason for this is to be found in Fig. 7, where it is shown that the Na content of the blood, and therefore presumably of the extracellular fluid, decreases during starvation, and this may be enough to counterbalance the small increase in the actual proportion of the extracellular fluid and keep the net Na constant for a time.

In mammals it is well known[27] that the water content of tissues diminishes during development, and that this change is accompanied by a fall in the concentrations of the extracellular ions Na and Cl. In man[27] and rabbit[48] a high proportion of extracellular space has been demonstrated histologically in foetal tissue, which decreases with growth.

In fish, a decrease in water content with increase in size has been reported for *Oncorhynchus gorbuscha*[49] and *Eptatretus stouti*.[50] However, although this seems to be a clear correlation between size, decrease in water (= decrease in extracellular fluid) and Na, it does not seem to explain the decrease in Na shown in well nourished cod of increasing size (Table III) in which the water content does not vary appreciably—certainly not enough to account for the marked change in ion concentration. The changes in Na, water and size reported in *Oncorhynchus gorbuscha*[49] were shown to occur in extremely small fish (less than 7·5 cm long) and it seems possible that here, and in the foetal mammals described, the muscle is at the stage of growing by multiplication of cells, not enlargement of existing cells. The cod in the present experiment are well past this stage, which probably occurs only if they are less than 25 cm long.[1]

Why then should the Na decrease with growth? In the first paper in this series[1] it was shown that the muscle cells steadily increased in diameter during growth of the fish, and it is reasonable to suppose that, as the number of expanding cells per g of tissue decreases, so the proportion of cell surface will decline, and so will the predominantly extracellular ions.

Finally, the work in this paper shows that connective tissue in the form of myocommata has the relatively high Na and low K contents characteristic of extracellular space. Since the proportions of myocommata to myotomes vary in different parts of the fillet, the myotomes must be dissected free from myocommata if meaningful results are to be obtained.

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Torry Research Station,
Aberdeen

References

1. Love, R. M., *J. Sci. Fd Agric.*, 1958, **9**, 195
2. Love, R. M., *J. Sci. Fd Agric.*, 1958, **9**, 199
3. Love, R. M., *J. Sci. Fd Agric.*, 1958, **9**, 617
4. Love, R. M., & Robertson, I., *J. Sci. Fd Agric.*, 1967, **18**, 217
5. Robertson, I., Love, R. M., & Cowie, W. P., *J. Sci. Fd Agric.*, 1967, **18**, 563
6. Love, R. M., *Nature, Lond.*, 1960, **185**, 692
7. Thurston, C. E., & MacMaster, P. P., *Fd Res.*, 1960, **25**, 229
8. Thurston, C. E., *Comml Fish. Rev.*, 1958, **20** (8), 21
9. Thurston, C. E., & Groninger, H. S., *J. agric. Fd Chem.*, 1959, **7**, 282
10. Thurston, C. E., *J. Fish. Res. Bd Can.*, 1962, **19**, 39
11. Kruchakova, F. A., *Trudy karadah. Biol. Sta., Nauk SSR*, 1952, (12), 111
12. Black, E. C., Connor, A. R., Lam, K.-C., & Chiu, W.-G., *J. Fish. Res. Bd Can.*, 1962, **19**, 409
13. Fraser, D. I., Dyer, W. J., Weinstein, H. M., Dingle, J. R., & Hines, J. A., *Can. J. Biochem.*, 1966, **44**, 1015
14. Amano, K., Bito, M., & Kawabata, T., *Bull. Jap. Soc. sci. Fish.*, 1953, **19**, 487
15. Nagayama, F., *Bull. Jap. Soc. sci. Fish.*, 1961, **27**, 1014
16. Wood, J. D., *J. Fish. Res. Bd Can.*, 1959, **16**, 755
17. Ironside, J. I. M., & Love, R. M., *J. Sci. Fd Agric.*, 1958, **9**, 597
18. Amoore, J. E., Bartley, W., & van Heyningen, R., *Biochem. J.*, 1959, **72**, 126
19. Rodeck, H., *Klin. Wschr.*, 1957, no. 35, 690

20. Jansen, H. H., *Virchows Arch. path. Anat. Physiol.*, 1961, **334,** 510
21. Sturner, W. Q., *Lancet*, 1963, (i), 807
22. Sturner, W. Q., & Gantner, G. E., *Am. J. clin. Path.*, 1964, **42,** 137
23. Love, R. M., *Nature, Lond.*, 1962, **196,** 593
24. Manery, J. F., In 'Mineral Metabolism', Ed. Comar & Bronner, 1962, 551, Academic Press, N.Y.
25. Hvidberg, E., Jensen-Holm, J., & Langgård, H., *Acta pharmac. tox.*, 1963, **20,** 131
26. Mori, K., & Duruisseau, J.-P., *Can. J. Biochem. Physiol.*, 1960, **38,** 919
27. Dickerson, J. W. T., & Widdowson, E. M., *Biochem. J.*, 1960, **74,** 247
28. Kravis, E. M., & Kare, M. R., *Poult. Sci.*, 1960, **39,** 13
29. Thurston, C. E., *J. Fd Sci.*, 1961, 26, 495
30. McBride, J. R., & MacLeod, R. A., *J. Am. diet. Ass.*, 1956, **32,** 636
31. MacLeod, R. A., Jonas, R. E. E., & McBride, J. R., *Can. J. Biochem. Physiol.*, 1958, **36,** 1257
32. Kordyl, E., *Pr. morsk. Inst. ryb. Gdyni*, 1951, no. 6, 145. Cited in *World Fish Abs.* **4,** no. 6, UDC 637.502.7
33. McBride, J. R., MacLeod, R. A., & Idler, D. R., *J. Fish. Res. Bd Can.*, 1959, **16,** 679
34. Greene, C. W., *J. biol. Chem.*, 1919, **39,** 435
35. Sutton, A. H., *Comp. Biochem. Physiol.*, 1968, **24,** 149
36. Thurston, C. E., & Newman, H. W., *Fish. Ind. Res.*, 1962, **2,** 15
37. Templeman, W., & Andrews, G. L., *J. Fish. Res. Bd Can.*, 1956, **13,** 149
38. Thurston, C. E., *J. agric. Fd Chem.*, 1961, **9,** 313
39. Meyer, D. K., Westfall, B. A., & Platner, W. S., *Am. J. Physiol.*, 1956, **184,** 553
40. Thurston, C. E., Stansby, M. E., Karrick, N. L., Miyauchi, D., & Clegg, W. C., *Fd Res.*, 1959, **24,** 493
41. Manery, J. F., & Hastings, A. B., *J. biol. Chem.*, 1939, **127,** 657
42. Robinson, J. R., In 'Mineral Metabolism', Ed. Comar & Bronner, 1962, 205. Academic Press, N.Y.
43. Manery, J. F., *Physiol. Rev.*, 1954, **34,** 334
44. Simon, S. E., Shaw, F. H., Bennett, S., & Muller, M., *J. gen. Physiol.*, 1957, **40,** 753
45. Conway, E. J., *Physiol. Rev.*, 1957, **37,** 84
46. Robertson, J. D., *J. exp. Biol.*, 1961, **38,** 707
47. Love, R. M., *J. Cons. int. Explor. Mer.*, 1962, **27,** 34
48. Mezincescu, M. D., & Strugali, H., *Acad. Rep. pop. Romîne, Inst. Biochem., Stud. Cercetări Biochim.*, 1958, **1,** 141. Cited in Chem. Abstr. 1959, **53,** 11574
49. Parker, R. R., & Vanstone, W. E., *J. Fish. Res. Bd Can.*, 1966, **23,** 1353
50. Munz, F. W., & Morris, R. W., *Comp. Biochem., Physiol.* 1965, **16,** 1

NUTRITIONAL EVALUATION OF SEED PROTEINS OF *ACTINODAPHNE HOOKERI*

By N. VENKATESAN and D. V. REGE

The possibility of utilisation of pisa meal, which remains after defatting of *Actinodaphne hookeri* seeds, as a potential protein source in foods and feeds has been investigated. Rat growth experiments with half of the dietary protein derived from the meal were carried out, using casein as standard protein for comparison. Reasonably good correlation between chemical data and biological performance was observed. The meal, when made nutritionally adequate by supplementation with methionine and lysine, was capable of supporting normal growth and maintaining other physiological functions in rats. Feeding the rats with pisa meal for 8–10 weeks did not reveal any abnormal biochemical lesion indicative of the onset of toxic action.

Introduction

The residues left after the removal of oil from several oilseeds, and the proteins isolated from such residues, either as such or after suitable processing, find use in livestock feeds, human diet and industrial applications.[1] A large variety of oilseed plants grow in Indian forests. Several of these have been termed 'inedible' ,since they contain toxic, bitter or astringent factors. The oils from some of these have been used in the soap industry, but the residual meals are not utilised because of lack of information on their toxicity and nutritional performance. Results of systematic studies on the chemical composition and nutritive evaluation of one such residue, pisa meal, obtained after the removal of fat from the seeds of *Actinodaphne hookeri*, are reported here.

Experimental

Expeller-pressed pisa meal was extracted with petroleum ether (boiling range 66–69°) in a Soxhlet extractor for 8 h to remove residual fat and air-dried. Nitrogen in pisa meal and casein was estimated by the Kjeldahl method[2] and factors of 6·25 and 6·38 were used respectively to arrive at crude protein content. The protein content of casein was 90% and that of pisa meal ranged from 36 to 42%.

10 g portions of the meal, in triplicate, were hydrolysed by refluxing with 10 volumes of 6 N-HCl for 18 h, and essential amino acids, except tryptophan, were estimated by standard microbiological procedures.[3]

Animal experiments

The meal used was either untreated or heat-treated. No external moisture was added to the meal prior to autoclaving. The meal which had an initial moisture content of 6–7% was autoclaved at 121° for ½ h and then dried in an air-drier at 40–50° for 2 h. The protein content of all the diets was 15%. All the experimental diets derived 50% of the protein from casein and the rest from pisa meal. The slightly bitter taste of the meal was masked by incorporating sucrose to the extent of 40% in the diets, in order to make the rations acceptable to rats.

The experiments were designed to assess the protein efficiency of the meal as well as to ascertain any possible toxic effects caused during prolonged feeding with it. The percentage essential amino acid (EAA) content of each diet was computed from the EAA compositions of casein[4] and pisa protein (Table I). This was then compared with the minimum EAA requirements for growing rats,[5] and in some diets the deficient amino acids, methionine and lysine, were added in amounts sufficient to make good the deficiencies (0·05 and 0·08% respectively on the basis of diet). The compositions of the diets are presented in Table II.

The rations also contained the following per 100 g: vit. B_{12}, 0·05 mg; vit. B_2, 2 mg; biotin, 0·06 mg; p-ABA, 2 mg 2 mg; vit. K, 1 mg; niacin, 4 mg; vit. B_1, 1 mg; calcium pantothenate, 10 mg; pyridoxine, 1 mg; inositol, 80 mg; folic acid, 1·1 mg and choline chloride, 100 mg. In addition vitamin A, 600 I.U., vitamin D_2, 10 I.U. and α-tocopherol, 3·0 mg were administered weekly to each rat.

Weanling laboratory-bred male rats of Wistar strain, of initial weight 60–70 g, were divided into groups of eight and housed in individual cages. Food and water were given *ad lib*. Records of weekly weight gain and daily food intake were maintained for an experimental period of 8–10 weeks and from these, protein efficiency ratio (PER) (g weight gain/g protein consumed) was calculated.

On termination of the growth period, the animals were dissected under ether, blood was drawn from the inferior vena cava and citrated and organs were quickly excised, washed with ice-cold distilled water, blotted and weighed. Liver was homogenised in distilled water in the cold in a Potter-Elvehjem type homogeniser.

TABLE I

Essential amino acids in pisa meal protein

Amino Acid*	g/16 gN
Arginine	8·4
Histidine	2·3
Isoleucine	2·9
Leucine	5·4
Lysine	4·1
Methionine	2·6
Phenylalanine	3·8
Threonine	2·9
Valine	4·4

* Calculated to 16 g nitrogen

Estimations

Total nitrogen in the liver homogenate and non-protein nitrogen in de-proteinised homogenate were determined by micro-Kjeldahl digestion and Nesslerisation.[6] Protein was obtained by difference. Liver xanthine oxidase activity was assayed manometrically in a Warburg apparatus according to the method of Dhungat & Sreenivasan,[7] and catalase activity was assayed colorimetrically.[8] Haemoglobin estimation was carried out by the acid haematin method[9] and percentage haemoglobin was calculated using the factor determined by Wong's iron estimation method.[10]

Results and Discussion

The growth patterns and tissue analyses of rats fed the different rations are given in Tables III and IV. For the purpose of comparison, the PER of the control diet was taken to be 100. Supplementation of untreated meal with methionine and lysine raised the PER (69–71·4%) almost to that of the control (90–96·9%). Autoclaving the meal reduced the PER of the raw meal. Supplementation of the autoclaved meal increased the PER which is still lower than that of the supplemented untreated meal. In both experiments, the food intakes of all the groups of animals were of similar order. No significant difference in any other parameter was observed.

Protein deficiency is known to adversely affect the activities of several enzymes in liver.[11,12] The effect on liver nitrogen and plasma proteins is less marked.[13,14] A high degree of correlation between liver xanthine oxidase activity and dietary protein changes has been observed, and the xanthine oxidase activity in liver has been proposed as a criterion of dietary protein quality.[15,16] However, in the present study, no significant differences in liver xanthine oxidase activity have been observed (Table III). The activities of liver glutamic–pyruvic and glutamic–oxalacetic transaminases of rats fed, on casein, yeast hydrolysate or yeast hydrolysate supplemented with methionine, cystine and lysine were in the same range, even though the PER values varied from 0·42 to 1·95.[15] Caution must be exercised in attempts to correlate enzymic activities with dietary protein quantity and quality, since other factors appear to have some effect. In the literature there are conflicting reports on the lability of tissue catalase levels to dietary protein changes, and these have been summarised by Knox *et al.*[12] Pisa meal constituents in diet do not exert any deleterious effect on the synthesis or activity of a typical porphyrino-protein, catalase in liver (Table III). It is possible that the amounts of lysine and methionine are

TABLE II

Percentage composition of diets

Ingredients	Control	Unsupplemented		Supplemented	
		untreated	autoclaved	untreated	autoclaved
Casein (90% protein)	16·70	8·35	8·35	8·35	8·35
Pisa meal	—	18·75[a]	20·90[b]	18·75	20·90
DL-Methionine	—	—	—	0·10	0·10
L-Lysine monohydrochloride	—	—	—	0·10	0·10
Maize starch	31·30	20·90	18·75	20·70	18·55

Ingredients common to all diets: vitaminised sucrose, 10%; sucrose, 30%; salt misture (U.S. P. No. XIV), 4%; sesame oil, 8%

[a]40% protein [b]36% protein

TABLE III

Effects of pisa protein on growth and metabolism of experimental rats

	Control group	untreated pisa meal	
		unsupplemented	supplemented
Weight gain for 10 weeks (g)	161 ± 21[a]	135 ± 13	175·4 ± 14·8
Food intake for 10 weeks (g)	737 ± 78	866 ± 82	830 ± 43·3
Protein efficiency ratio (PER)	1·454 (100%)[b]	1·038 (71·4%)	1·409 (96·9%)

[a] Mean ± SD
[b] Figures in parentheses are relative and are based on the value of 100 for control diet

TABLE IV

Comparison of raw and autoclaved pisa meals with respect to nutritive value

	Control	Unsupplemented		Supplemented	
		raw	autoclaved	raw	autoclaved
Weight gain for 8 weeks, g	197·6 ± 13·6[a]	137·3 ± 16·35	125·6 ± 28·9	185·9 ± 15·69	175·1 ± 12·93
Food intake for 8 weeks, g	693·2 ± 71·9	662·9 ± 36·2	722·4 ± 73·6	739·1 ± 44·6	769·0 ± 63·8
Protein efficiency ratio (PER)	1·95 (100%)[b]	1·34 (69%)	1·18 (60·8%)	1·75 (90%)	1·55 (79·8%)

[a] Mean ± SD
[b] Figures in parentheses are relative and are based upon a value of 100 for the control diet

sufficient to meet the animal's needs for enzyme synthesis but just fall short of the requirements for maintaining a high growth rate.

Gain in bodyweight, in some cases, depends more upon deposition of fat than on increase of body protein.[16] Since total nitrogen, non-protein nitrogen and protein nitrogen in liver, and the weights of different organs are of the same order for different groups, the observed bodyweight increase in the present study is indicative of tissue protein synthesis and not a deposition of body fat.

The possibility of variations in the quantity and quality of amino acids available simultaneously to the animal must be taken into consideration, because these can arise from differences in amino acid make-up and in *in vivo* hydrolysis rates of casein and pisa meal protein. Since the food intakes for the different groups were of the same magnitude and no fatty globules in liver or growth depression were observed, any serious imbalances or antagonisms among amino acids were ruled out.

Several leguminous foodstuffs and oilseed meals have been shown to contain heat-labile toxins, e.g., trypsin inhibitors, haemagglutinins and aflatoxins.[17–20] Suitable heat treatment destroys or inactivates these, thereby resulting in enhanced nutritive value. On the other hand, severe heating is known to exert deleterious effects on the nutritional value of proteins.[21] Autoclaving pisa meal at 121° for ½ h depresses the growth rate of rats fed on it. This could be explained on the basis of accentuation of the already existing amino acid deficiency in the presence of naturally occurring carbohydrate-like materials in the meal. Apart from this difference in growth response between the raw and autoclaved meals, no perceptible changes in other parameters such as liver nitrogen reserves and enzyme levels, blood haemoglobin and size of organs in relation to bodyweight were detected.

Visual examination of organs of the rats fed untreated meals or autoclaved meals did not indicate any metabolic disorder such as liver necrosis or fatty infiltration usually caused by foreign toxic agents such as ethionine, t-butyl-methylnitrosamine and aflatoxins.[22,23]

In the light of these observations, it was concluded that pisa meal does not possess any toxic or anti-nutritional factor (heat-labile or heat-stable) and that when supplemented with amino acids, it can support and maintain normal growth and metabolic pattern of rats.

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Dept. of Chemical Technology,
University of Bombay,
Matunga, Bombay 19, India

References

1. Altschul, A. M., 'Processed plant protein foodstuffs', 1958 (Academic Press, New York)
2. 'Methods of analysis', Ass. Off. Agric. Chem. Washington, D.C., 9th Edition, 1960
3. Barton-Wright, E. C., 'Microbiological assay of vitamin B-complex and amino acids, '(Sir Issac Pitman and Sons Ltd., London), 1952
4. Block, R. J., & Weiss, K. W., 'Amino Acid Handbook', (C. C. Thomas, Illinois), 1956, p. 342
5. Block, R. J., & Weiss, K. W., 'Amino Acid Handbook', (C. C. Thomas, Illinois), 1956, p. 164
6. Johnson, M. J., *J. biol. Chem.*, 1941, **137,** 575
7. Dhungat, S. B., & Sreenivasan, A., *J. biol. Chem.*, 1954, **208,** 845
8. Bhuvaneswaran, C., Sreenivasan, A., & Rege, D. V., *Enzymologia*, 1961, **23,** 186
9. Cohen, B., & Smith, A. H., *J. biol. Chem.*, 1919, **39,** 489
10. Wong, S. Y., *J. biol. Chem.*, 1928, **77,** 409
11. Awapara, J., *J. biol. Chem.*, 1953, **200,** 537
12. Knox, W. E., Auerbach, V. H., & Lin, E. C. C., *Physiol. Rev.*, 1956, **36,** 164
13. Litwack, G., Fatterpaker, P., Williams, J. N., jun., & Elvehjem, C. A., *J. Nutr.*, 1954, **52,** 187
14. Chow, B. F., Seeley, R. D., Allison, J. B., & Cole, W. H., *Arch. Biochem.*, 1948, **16,** 69
15. Rastogi, M. K., & Krishna Murti, C. R., *Indian J. exp. Biol.*, 1964, **2,** 143
16. Allison, J. B., *Physiol. Rev.*, 1955, **35,** 664
17. Liener, I. E., in 'Processed plant protein foodstuffs', Ed. Altschul, A. M., (Academic Press, New York), 1958
18. De Muelanaere, H. J. H., *Nature, Lond.*, 1965, **206,** 827
19. Lancaster, M. C., Jenkins, F. P., & Philip, J. M., *Nature, Lond.*, 1961, **192,** 1095
20. Barner, J. M., & Butler, W. H., *Nature, Lond.*, 1964, **202,** 1016
21. Rice, E. E., & Benk, J. F., *Adv. Fd Res.*, 1953, **4,** 233
22. Farber, E., Shull, K. H., Villa-Trevino, S., Lombardi, B., & Thomas, M., *Nature, Lond.*, 1964, **203,** 34
23. Clifford, J. I., & Rees, K. R., *Nature, Lond.*, 1966, **209,** 312

JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE

ABSTRACTS

JULY, 1968

1.—AGRICULTURE AND HORTICULTURE

General: Soils and Fertilisers

Sieve analysis of particles smaller than forty-four microns. M. J. Nuckolls and R. K. Fuller (*Soil Sci.*, 1966, **102**, 292–295).—Small samples of particulate matter resulting from volcanic or other explosions and having dia. $<44\ \mu$ were separated into five discrete size-fractions in apparatus which included Micro-Mesh sieves and an ultrasonic shaker. The sample (1–10 g) is wet sieved through a 325-mesh (44 μ) sieve by a mechanical shaker. The portion $<44\ \mu$ is washed on to a 40-mesh sieve and shaken (3 min.) ultrasonically, particles passing the sieve being fractionated similarly through 30, 20 and 10 μ sieves, and each sieve and its retained particles is dried and weighed. A. G. POLLARD.

Volume changes in bulk density samples [of soils]. W. F. Miller (*Soil Sci.*, 1966, **102**, 300–304).—Core samples of soils generally used for bulk density measurements in moist soils may lose up to 30% of their vol. during oven drying. Relationships between this change in vol., variations in bulk density and independent variables: (*a*) wt.-% soil moisture at sampling, (*b*) % org. matter, % clay, % sand and initial bulk density were studied. Positive correlation between (*a*) and the vol. change and a negative relationship with variation in bulk density were found. Vol. changes were negligible in the A-horizon, averaged 11% in B_2 horizons, 13% in B_3 and 17% in C-horizons. A method is described for adjusting bulk density samples collected above or below field capacity to a common value at field capacity. A. G. POLLARD.

Experiment on suction drain from an ideal soil. T. Tabuchi (*Soil Sci.*, 1966, **102**, 329–332).—Glass beads of dia. 0·06–0·07 cm were packed by vibration in a beaker of water. The packed beads were photographed and the distribution of the pore necks was calculated. The horizontal and vertical suction drains were thus established. In the horizontal measurement the data produced experimental curves of similar shapes but there were some discrepancies between practical and theoretical values. In the vertical measurements, a water distribution curve in the bead column was similar to that of the capillary moisture distribution curve. With the most open packing of the beads, theoretical and experimental values were very similar but a basis for actual comparison was not found. A. G. POLLARD.

Vapour transfer in soil due to air turbulence. D. A. Farrell, E. L. Greacen and C. G. Gurr (*Soil Sci.*, 1966, **102**, 305–311).—An induction-type diaphragm pressure transducer was used to measure differences between air pressures at any two points within the soil or in air above the soil. Data obtained suggests that the conclusions reached by Fukuda (*ibid.*, 1955, **79**, 249) are erroneous. Assuming that air pressures can be described as a train of sinusoidal pressure waves it follows that (*a*) the soil may be assumed infinite in depth if its thickness is of the same order as that of the wavelength of the pressure waves, (*b*) air-movement within the soil is many times greater than was previously assumed, and (*c*), for a wind speed of 15 m.p.h. surface air can penetrate coarse soil and crop residue mulches to a depth of several cm, approx. 10 times that suggested by Fukuda. If gaseous diffusion is assumed to be dependent mainly on the random macroscopic velocity of the soil air, the effective diffusion coeff. for coarse mulches can be as much as 100 times the mol. diffusion. The mathematical basis of these conclusions is presented. In quant. studies of the efficiency of mulches the influence of wind must be considered.
A. G. POLLARD.

Adsorption of water and of electrolyte solutions by kaolin clay suspensions. L. A. G. Aylmore and J. P. Quirk (*Soil Sci.*, 1966, **102**, 339–345).—Homoionic, air-dried clays were ground and compressed into small cores, which were equilibriated with saturated salt solutions providing a range of constant water-vapour pressures. Equilibrium with the cores was normally reached in 2–3 days and appropriate data were taken in 5 days. The swelling of the cores was examined in relation to hydrostatic suction, electrolyte concn., sp. surface area and exchangeable cation capacities of the clays. Lack of correlation between solution contents and the physicochemical characteristics of the clays was notable. Swelling seems to be governed by variations in the structural strength of the clay matrix. A. G. POLLARD.

Comparison of mulches for erosion control and grass establishment on light soil. J. B. Beard (*Q. Bull. Mich. St. Univ. agric. Exp. Stn.*, 1966, **48**, 369–376).—Various methods of protection against erosion on sloping land are compared. Covers of straw-asphalt, straw alone and straw with net covering were superior to several manufactured mulches. The latter materials gave useful control in the early part of the season, but later did not cause sufficient retention of soil water to permit the establishment of a grass cover. Mineral and org. sods, gave similar control of erosion. A. G. POLLARD.

Chemical properties of a New Jersey podzol as affected by leaching with various agents. M. Levesque and W. J. Hanna (*Soil Sci.*, 1966, **102**, 333–338).—The abilities of fulvic acid and of EDTA to mobilise soil constituents and so produce changes in a soil profile, are examined. A glacial drift pasture soil was leached, over 10 weeks, with (*a*) water (*b*) 0·0005 M-EDTA or (*c*) 0·005 M-fulvic acid extracted from soil. ^{32}P and ^{59}Fe were previously added to the A_2 horizon. Movements of ^{32}P and ^{59}Fe were observed. Differences in leaching solutions caused marked differences in the amounts of Fe moved. Water moved less of all elements measured than did the other reagents. The larger amounts of Fe moved by EDTA were not accompanied by larger amounts of P from the A_2 horizon. (cf. *Can. J. Soil Sci.*, 1966, **46**, 53). A. G. POLLARD.

Complexes of montmorillonite with primary, secondary and tertiary amides. II. Coordination of amides on the surface of montmorillonite. S. A. Tahoun and M. M. Mortland (*Soil Sci.*, 1966, **102**, 314–321: cf. *ibid.*, **102**, 248).—Complexes formed by amides with metal-ion-saturated montmorillonite, result from a co-ordinate bond between the O atom of the amide and the exchangeable ion. Observations on the strength of this bond in relation to the order of the amides (primary < secondary < tertiary) and to the nature of the exchangeable ion, are made by i.r. spectroscopy. The possible formation of similar complexes with peptides and proteins is discussed. A. G. POLLARD.

Phosphorus status of some Nigerian soils. W. O. Enwezor and A. W. Moore (*Soil Sci.*, 1966, **102**, 322–328).—The P contents of a no. of forest and of savanna soils are examined. Both types showed low values. In savanna areas the upper 0–6 in. layer is important since the practice of 'shifting cultivation' is general. In the forest areas the annual cycle of vegetation maintains a small but consistent amount of available P. For this reason moderately leached soils have an accumulation (though small) of total P in the surface layers. Org. P constituents constitute 43 and 28·6% respectively, of the total P in the 0–6 and 6–12 in. layers in forest soils; in savanna soils, the corresponding values were 18 and 10%. Over half the extractable inorg. P was present as Fe phosphates, the remainder being approx. equal parts of Al and Ca phosphates. Sorption capacity for P was very high in heavy-textured soils but there was little evidence of P fixation. In forest soils org. P accumulated in the 0–2 in. layer. A. G. POLLARD.

Comparison of two methods for determining organic phosphorus in some Nigerian soils. W. O. Enwezor and A. W. Moore (*Soil Sci.*, 1966, **102**, 284–285).—Comparison is made of two methods for determining org. P in soils, (*a*) acid and alkaline extraction

followed by conversion into inorg. P and (*b*) conversion of all P into inorg. P by ignition of the soil at 550° and extraction with 0·2 N-H_2SO_4, the initial inorg. P being deducted in each case. Published variations of the two methods are discussed. The alkali extraction method is preferred. A. G. POLLARD.

Cleavage of humic acids by *Penicillium frequentans*. E. A. Paul and S. P. Mathur (*Pl. Soil*, 1967, **27**, 297–299).—The content of functional groups in undegraded humic acids was compared with that of humic acids which had been partially degraded by *Penicillium frequentans*. The degradation did not result in any change in -COOH groups, but resulted in a considerable increase in -OH, probably derived from hydrolysis of ether bonds. A. H. CORNFIELD.

Isolation of protein from humic acid extracted from soil. P. Simonart, L. Batistic and J. Mayaudon (*Pl. Soil*, 1967, **27**, 153–161).—Protein was isolated from humic acid extracted from three soil types. The protein fraction contained 14·8% N and yielded 20 different NH_2-acids on hydrolysis with 6 N-HCl. The fraction showed max. absorption at 260–280 mμ and had a small electronegative charge. A. H. CORNFIELD.

Use of molasses and distillery slops with sulphur for reclaiming sodic and saline-sodic soils from Puerto Rico. R. P. Escolar (*J. Agric. Univ. P. Rico*, 1967, **51**, 55–65).—Crop yields on saline-sodic and sodic clay soils were improved by application of molasses alone or with S and by distillery slops with S. Hydraulic conductivity was markedly increased by the slops and molasses treatments combined with S. A. H. CORNFIELD.

Chemical changes induced by autoclaving and γ-irradiation of soils and comparison of soil sterilised by the two methods for microbial colonisation experiments. P. O. Salonius, J. B. Robinson and F. E. Chase (*Pl. Soil*, 1967, **27**, 239–248).—Irradiation of a clay soil with a sterilising dose of γ-rays (3 megarads) had less effect than did steam sterilisation (121° for 1 h) in increasing water-sol. org. matter and major and trace metal ions. Soil NO_3^- content was little affected by either treatment. Radiation released greater amounts of NH_4^+ than did autoclaving when the soil was moist, but the reverse was true when the soil was wet. Radiation decreased, whilst autoclaving increased, water-stable aggregation. *Arthrobacter* and *Pseudomonas* grew better in irradiated than in autoclaved soil. A. H. CORNFIELD.

Microbiological aspects of soil structure. II. Soil aggregation by the extracellular polysaccharide of *Lipomyces starkeyi*. D. Jones and E. Griffiths (*Pl. Soil*, 1967, **27**, 187–200).—Artificial soil aggregates incubated with glucose showed marked increase in water stability. The yeast *Lipomyces starkeyi* increased in abundance with increasing aggregation. Whole-cell prep. and extracellular polysaccharides of the organism were also effective aggregating agents. A. H. CORNFIELD.

Antibiotic resistance of soil bacteria. I. Antibiotic resistance of bacteria from rhizosphere and non-rhizosphere soils. P. J. Harris and M. Woodbine (*Pl. Soil*, 1967, **27**, 167–171).—Numerous isolates from four rhizosphere and eight non-rhizosphere soils were examined for resistance to seven antibiotics. There were marked differences in the overall levels of antibiotic resistance found between different soils. For all soils there was no correlation between antibitotic resistance and org. matter or pH. Rhizosphere populations were more resistant than their corresponding non-rhizosphere populations for four of the antibiotics and three of the four soils, but for the soils and antibiotics as a whole there were no consistent differences in the resistance of the two types of population. A. H. CORNFIELD.

Interrelations of soil micro-organisms and mulberry. I. Phytohormone production by soil and rhizosphere bacteria and their effect on plant growth. V. N. Vasantharajan and J. V. Bhat (*Pl. Soil*, 1967, **27**, 261–272).—About 13% of the mulberry rhizosphere and 10% of the non-rhizosphere isolates produced phytohormones in cultures, the rhizosphere isolates being more active in hormone synthesis. Soaking mulberry stem cuttings in culture filtrates of phytohormone synthesisers hastened their rooting. Culture filtrates of many isolates, hormone producers or not, stimulated or inhibited the growth of shoots and/or roots of mulberry. About 20% inhibited the germination of mulberry seeds. A. H. CORNFIELD.

Urease activity in soils of northern New South Wales, Australia. J. W. McGarity and M. G. Myers (*Pl. Soil*, 1967, **27**, 217–238).—The urease activity of pasture soils was greater in Krasnozem and Chocolate great soil groups than in red-brown earths and podzols. There were weak positive correlations between urease activity and both soil org. C and pH, and urease activity was not related to either soil colour or texture. Extracellular urease activity was highly correlated with viable urease activity. Extracellular activity was significantly higher than viable activity in near-neutral than in acid soils. The nature of the pasture type did not affect the proportion of the two types of activity. In general urease activity was similar between winter and spring samplings. The level of urease activity in surface pasture soils is considered to be adequate for rapid hydrolysis of the usual concn. of urea applied in urine or as fertiliser. A. H. CORNFIELD.

Applicability of a new fertiliser-yield relation. B. G. Capó (*J. Agric. Univ. P. Rico*, 1967, **51**, 97–120).—The equation suggested is $Y = A/[1+B(X-C)^2]$, where Y = crop yield, X = quantity of fertiliser applied, A = max. yield obtainable with optimum fertiliser application (C), and B = an index of the variability of crop yield as the quantity of fertiliser applied differs from the optimum application C. The equation satisfactorily fitted fertiliser–yield data in 34 experiments in Puerto Rico on studies of the effects of N, P, K, Ca, and Mg on yields of sugar cane and maize, even where equations suggested by other workers were not satisfactory. A. H. CORNFIELD.

Sulphur content of Nigerian manures. O. L. Oke (*Expl. Agric.*, 1967, **3**, 322–326).—Total and mineralisable S, sol. SO_4^{2-}, and total N are presented for faeces of chick, cow, pig, goat, horse and also for compost and farmyard manure. Chick and goat faeces were the highest in total S (dry basis). For all the materials total S was highly correlated with total N. A. H. CORNFIELD.

Foam plastic as a growth medium in hydroponics. M. Schwarz (*Pl. Soil*, 1967, **27**, 289–291).—Providing polystyrene foam pellets were washed with water for 3–4 weeks before use (to remove HCHO) they successfully replaced the lower two-thirds of gravel in hydroponic beds. A. H. CORNFIELD.

Plant Physiology, Nutrition and Biochemistry

Crystalline silica in plants. C. Sterling (*Am. J. Bot.*, 1967, **54**, 840–844).—Cryst. SiO_2 was found in *Fragaria* leaves, *Equisetum* shoots and in diatomite and tabaschir. Amorphous SiO_2 was present in each of these and X-ray reflections of α-quartz, low tridymite or α-cristobalite were found in nearly all specimens. Unidentified cryst. reflections were observed, notably in the SiO_2 isolated from *Fragaria* leaves and diatomite. A. G. POLLARD.

Nucleotides of sugarcane: trichloracetic acid-soluble and protein-bound nucleotides of sugarcane meristem. A. G. Alexander (*J. Agric. Univ. P. Rico*, 1967, **51**, 210–227).—Uridine, adenosine and cytidine were positively identified, uracil was possibly present, whilst the presence of guanosine and cytosine was doubtful. Glucosamine was present in trichloracetic acid extracts. Uridine diphosphate-glucose and ribose or a ribose deriv. were tentatively identified. A. H. CORNFIELD.

Nucleotides of sugarcane: increased nucleotide content of leaves due to nutritional stress. A. G. Alexander (*J. Agric. Univ. P. Rico*, 1967, **51**, 228–237).—High levels of sucrose and total nucleotides appeared in sugarcane leaf tissue receiving low levels of NO_3^-, P, K, or Ca. Nucleotide increase was greatest with low-Ca and low-N and least with low-K. Nucleotide prep. from low-N and low-P plants contained high levels of reducing sugar and inorg. and org. P, whilst low-K and low-Ca plants were high in sucrose without accumulating org. P. Adenosine and cytidine were present in all samples. Guanosine was found only in N-, P-, and K-deficient plants. A. H. CORNFIELD.

Effects of variable calcium and manganese on the enzyme nucleotide and nutrient constituents of sugarcane grown in sand culture. A. G. Alexander and G. Samuels (*J. Agric. Univ. P. Rico*, 1967, **51**, 238–249).—The effect of 0 and 10 mequiv. per l of Ca and 0, 10, and 100 ppm Mn in the nutrient on sugarcane from 16 to 30 weeks of age was studied. Leaf Mn% was depressed and amylase action was retarded by high Mn only when Ca supply was low. High Ca greatly stimulated peroxidase. The treatments had little effect on the content of acid-sol. nucleotides. Sugar content was greatest in nucleotide prep. from plants receiving both high Ca and Mn, whilst P was highest in those from plants low in Ca. A. H. CORNFIELD.

Uptake and translocation of zinc by intact plants. L. R. Hawf and W. E. Schmid (*Pl. Soil*, 1967, **27**, 249–260).—Addition of 5 μM or more of Cu^{2+} or Cd^{2+} to the nutrient solution severely reduced uptake of Zn, but translocation of Zn from roots to tops was not much affected. Addition of Mn^{2+} did not affect Zn uptake until

30 μM or more of Mn^{2+} was added, when Zn uptake was reduced by about 50%. Mn^{2+} had little effect on translocation of Zn within the plant. A. H. CORNFIELD.

Nitrogen, phosphorus, and potassium contents of sugarcane leaf as related to part of the leaf and position on the plant. G. Samuels (*J. Agric. Univ. P. Rico*, 1967, **51**, 22–28).—The N% and P% of the leaf blade were higher than those of the leaf sheaf, but the reverse was true for K%. Leaf N% decreased only slightly with increasing leaf number (from the spindle leaf downwards), but leaf P% and K% decreased rapidly with increasing leaf number. Leaf N% and P% increased considerably from base to tip, but the reverse was true for leaf K%. A. H. CORNFIELD.

Variation in chemical composition of guava leaves as affected by position on the terminals. S. J. Rodriguez (*J. Agric. Univ. P. Rico*, 1967, **51**, 252–259).—The N, P, K, Ca, Mg, Na, Mn, Fe, Cu, B, Zn, and Al contents (%) in the leaf from the apex of the terminal to where it joined the stem are presented for fruiting and non-fruiting branches of guava. Results are discussed in relation to the most suitable leaf to be analysed to indicate the nutritional status of the tree. A. H. CORNFIELD.

Boron adsorption by plant roots. H. Tanaka (*Pl. Soil*, 1967, **27**, 300–302).—Dicotyledonous plants, which have a higher B requirement than do monocotyledonous plants, also showed a higher capacity for their roots to adsorb BO_3^{3-}. A. H. CORNFIELD.

Boron analysis in plant material. J. L. Paul and L. V. Smith (*Soil Sci.*, 1966, **102**, 353).—Comparison is made of the dry ashing method with that of Yoshida and Yoshida (*J. Sci. Soil and Manure, Tokyo*, 1965, **36**, 45) involving shaking the material with HCl and determining B in the extract. With different plant material, the concn. of HCl, and time of extraction needed to remove an amount of B equal to that determined by the ignition method, varied considerably. Probably B is present in different plant species in more than one form, having different solubilities. A. G. POLLARD.

Movement and fate of ^{14}carbon-labelled sorbitol in the apple tree and fruit. M. W. Williams, G. C. Martin and E. A. Stahly (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 20–24).—Sorbitol applied to the leaves, and to the fruiting and non-fruiting spurs moved rapidly in the tree and was converted to sugars, NH_2-acids and other org. compounds. Phloridzin became radioactive soon after labelled sorbitol was applied to vegetative tissue. Sorbitol was absorbed and translocated faster than sucrose and continued to move into apple fruit after watercore began to develop. A. H. CORNFIELD.

Non-volatile acids of green peas. R. McFeeters, S. H. Schanderl and P. Markakis (*Q. Bull. Mich. St. Univ. agric. Exp. Stn*, 1966, **48**, 417–420).—Freshly picked peas were frozen or heat processed within 2 h of picking. Subsequently, extracts of the peas were examined chromatographically and showed the presence of small amounts of galacturonic acid and pyrrolidone carboxylic acid (possibly formed during heating), fumaric and caffeic acids; larger proportions of citric, malic, succinic and phosphoric acids were also found. A. G. POLLARD.

Effect of drought and low temperature on leaf freezing points and on water-soluble proteins and nucleic acid content of sweet orange plants. M. Z. S. Ghazaleh and C. H. Hendershott (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 93–102).—When plants were exposed to low night temp. (4·5°) water-sol. bark proteins and leaf f.p. increased; the latter was not related to leaf water-sol. protein or nucleic acid content. Exposure of plants to drought did not affect water-sol. protein content of the leaves. Exposure of plants to drought or low night temp. increased RNA and DNA activities of the leaf tissue. A. H. CORNFIELD.

Seasonal changes in oxidation and phosphorylation in mitochondrial preparations from grapefruit. H. M. Vines and J. F. Metcalf (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 86–92).—The activity of mitochondrial prep. in oxidising intermediates of the citric acid cycle was low in prep. from immature fruit, but increased rapidly as the fruit approached maturity. Thereafter activity generally declined as the fruit matured and ripened. Changes in activity were correlated with changes in the acidity of the juice. A. H. CORNFIELD.

Purification and electrophoretic behaviour of sugarcane invertase. A. G. Alexander (*J. Agric. Univ. P. Rico*, 1967, **51**, 39–45).—A fair degree of sugarcane invertase purification was achieved by differential solubility, gel filtration, and paper electrophoresis. In electrophoresis invertases were quite mobile compared with contaminant proteins. Two distinct invertase areas were obtained free of contaminant protein, one enzyme bearing a positive and the other a negative charge. A. H. CORNFIELD.

Effect of ionising radiation and diphenylamine on glucose metabolism and membrane permeability in Cortland apples. M. Faust, B. R. Chase and L. M. Massey, jun. (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 25–32).—Irradiation of apple fruit with γ-rays, 2 weeks before commercial picking, altered the respiratory system of the apple from the Embden-Mayerhof-Parnas glycolitic pathway to the pentose phosphate pathway within 3 weeks of irradiation. The major mode of action of ionising radiation in preventing scald was probably to shift metabolic pathways toward those which provide more resistance to the apple. Radiation caused changes in membrane permeability, but these changes were not related to scald development. Dipping fruit in diphenylamine (2000 ppm) induced changes similar to those caused by radiation in regard to the pentose phosphate pathway, but it did not change the membrane permeability of the cells. A. H. CORNFIELD.

Chlorogenic acid, tyrosine, and bisulphite in enzymic blackening of potatoes. P. Muneta (*Am. Potato J.*, 1967, **44**, 236–240).—Warburg manometric tests were used to study the interaction of crude polyphenol oxidase from potatoes with chlorogenic acid, tyrosine, and $NaHSO_3$. $5{\cdot}4 \times 10^{-4}$ to 1×10^{-3} M-HSO_3^- inhibited tyrosine oxidation for 30–40 min., but did not inhibit chlorogenic acid oxidation. $1{\cdot}8 \times 10^{-2}$ M-HSO_3^- inhibited chlorogenic acid oxidation by about half and inhibited the oxidation of 3,4-dihydroxyphenylalanine to a greater extent. A. H. CORNFIELD.

Absorption and translocation of iodoacetic acid by foliage of two sweet orange varieties. J. H. Talton, jun., C. H. Hendershott and R. H. Biggs (*Proc. Am.. Soc. hort. Sci.*, 1967, **90**, 117–122).—Within 2 h of application to the leaf, COOH-^{14}C-labelled Cl_3COOH (I) was well distributed through the leaf in both varieties of sweet orange. Activity was concentrated in the leaf tissue adjacent to the area of application and in the vascular system of the leaf. Leaves of both varieties readily decarboxylated I and the rate of decarboxylation increased for at least 25·5 h after treatment. A. H. CORNFIELD.

Effects of chemical treatments on the rate of abscission of Hamlin orange explants. W. C. Wilson and C. H. Hendershott (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 123–129).—Of 33 chemicals and combinations tested 5×10^{-4} M-CI_3COOH was the most active in increasing abscission of orange fruit using explants, but 1% mannitol was also very effective. Cu^{2+}, Fe^{2+}, Fe^{3+}, $NaHSO_3$, CF_3COOH, and 6-phosphogluconic acid also increased abscission, whilst 2,4-D (10^{-4}-M), sucrose, hexose sugars, H_3BO_3 and naphthylacetic acid delayed abscission. A. H. CORNFIELD.

Extraction and assay of the antibiotic, phytoactin, from western white pine tissue. A. E. Harvey, S. O. Graham and L. D. Becker (*Phytopathology*, 1967, **57**, 99).—The fresh tissue is lyophilised to nearly zero moisture, desiccated and ground to particle size 40-mesh, extracted (2 h) with light petroleum, the residue then being extracted twice with acetone. The dried residue from the acetone extracts is dissolved in a little chilled (−50°) acetone and chilled light petroleum is added. The ppt. is dissolved in 95% ethanol and passed through a column of DEAE-Sulphadex A25. Phytoactin A remains on the resin when phytoactin B is eluted by ethanol. 95% ethanol + 0·10 M-HCl was used to elute phytoactin A. Each eluate was vac.-dried and the residue dissolved in ethanol and used to assay *Glomerella cingulata*. Phytoactin B in amounts 0·5 ppm in the treated tissue may thus be assayed but phytoactin A is difficult to detect in amounts < 250 ppm. A. G. POLLARD.

Influence of ultra-violet radiation on auxin-controlled plant growth. R. M. Klein (*Am. J. Bot.*, 1967, **54**, 904–914).—Plant systems producing or containing deficient, sufficient or excessive amounts of auxin were exposed to u.v. radiation (254 mμ) and subsequently showed altered growth capacities which could be ascribed to altered ability of the plant cells to respond to auxin. This effect of u.v. radiation was usually reversible by subsequent exposure to visible or near-visible radiation. A. G. POLLARD.

Effect of auxin on suckering in black locust, *Robinia pseudoacacia*. J. P. Sterrett and W. E. Chappell (*Weeds*, 1967, **15**, 323–326).—When black locust seedlings were decapitated both stump sprouting and root suckering occurred, but the presence of stump sprouts prevented root suckering. The influence of stump sprouts could be replaced by treating cut surfaces of root stubs with indole-acetic acid (IAA). When ^{14}C-labelled IAA was used, ^{14}C activity was concentrated where the majority of the root suckers normally would appear, and vigorous root suckering occurred when IAA was removed from the root stubs. A. H. CORNFIELD.

Effect of potassium gibberellate on fruit characteristics and flowering of the pecan. D. Sparks (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 61–66).—Application of 200 ppm K gibberellate (KGA) on 1st June induced some of the dormant buds on pecan trees to break and grow. Shuck wt. increased with concn. of KGA up to 400 ppm, whether applied early (June) or late (Aug.). Maturity was delayed and nut wt. increased with increasing KGA concn. (200–600 ppm) applied late. In the spring following treatment bud break was delayed slightly and catkin formation was inhibited where KGA had been applied early the previous year. A. H. CORNFIELD.

Factors influencing root formation in hardwood cuttings of fruit trees. R. F. Carlson (*Q. Bull. Mich. St. Univ. agric. Exp. Stn*, 1966, **48**, 449–454).—Hardwood cuttings from *Malus* and *Prunus* spp. were soaked for 24 h in a solution of 3-indole-butyric acid (**I**) (optionally 50 ppm) before placing in the rooting medium. Sweet cherry varieties responded notably to differences in rooting media, the preference being for well aerated mixtures. Vigorous cuttings of the current season's growth rooted most readily. Differences in concn. of **I** and period of treatment had varied effects on root induction. Controlled temp. (65–70° F) and uniform moisture content in the rooting medium were necessary for successful rooting and handling of the rooted cuttings should be minimal. Soaking in **I** of concn. >50 ppm tended to suppress rootings and brief dipping in more conc. solution (2000 ppm) gave better results. A. G. POLLARD.

Gibberellin-induced inhibition of floral initiation in fuchsia. R. M. Sachs, A. M. Kofranek and Shiow-Ying Shyr (*Am. J. Bot.*, 1967, **54**, 921–929).—A cultivar of *Fuchsia hybrida* (a long-day plant) reacts to gibberellin (*G*) which inhibits floral initiation. Dosages of *G* needed to cause inhibition (0·025 μg/plant) also promote increased stem elongation. Auxins, kinins and anti-auxins at concn. similar to that of *G* which inhibits floral initiation, did not disturb initiation but at 10–100-fold this dosage, inhibited both initiation and vegetative development. Inhibition by *G* was directly proportional to the dosage and inversely to the strength of the long-day induction (no. of long days). *G* was most effective when applied to the terminal bud rather than to mature leaves; it did not prevent floral initiation if applied after the translocation of the floral stimuli from the leaves. *G* was less effective if given later rather than earlier in the long-day induction period. The inhibitory period persists for several days; flower development and rate of bud growth were not affected. A. G. POLLARD.

Crops and Cropping

Systematic designs for spacing experiments. J. K. A. Bleasdale (*Expl. Agric.*, 1967, **3**, 73–85).—Two types of design are described in which the number of plants per unit area varied systematically within any one plot in the field, the plant arrangement being held constant. Any desired range of spacing can be covered in either design and the calculations necessary to establish specific sets of dimensions are given. A. H. CORNFIELD.

Variation in crop yields from year to year. J. E. Jackson (*Expl. Agric.*, 1967, **3**, 175–182).—Methods of determining the causes of year-to-year variation in yields are discussed. A. H. CORNFIELD.

Responses of cereals to fertilisers in Northern Nigeria. I. Sorghum. P. R. Goldsworthy (*Expl. Agric.*, 1967, **3**, 29–40).—On the basis of many field trials five fertiliser response areas were defined and estimates were made of the most profitable combinations and levels of P and N to use in each area. A. H. CORNFIELD.

Responses of cereals to fertilisers in Northern Nigeria. II. Maize. P. R. Goldsworthy (*Expl. Agric.*, 1967, **3**, 263–273).—In the south the main response was due to N and further north, with the exception of a small area in which the response was due almost entirely to P, both P and N were equal limiting factors. Estimates of the most profitable combinations of N and P to use have been obtained from quadratic response surfaces fitted to the yield data. A. H. CORNFIELD.

Crop rotation–date of sowing interactions affecting foot-rot in winter wheat. D. M. Huber (*Phytopathology*, 1967, **57**, 99).—The occurrence of *Cercosporella* and *Rhizoctonia* foot-rots diminished with later sowing between Aug. 1st. and Nov. 1st. Yields tended to increase with early sowing in absence of foot-rot but sowing prior to Sept. 1st. (Idaho, U.S.A.) intensified the rot regardless of the crop rotation adopted. A. G. POLLARD.

Nitrogen fertiliser timing for winter potatoes [in Arizona]. P. M. Bessey (*Am. Potato J.*, 1967, **44**, 214–217).—Yields of potatoes on an irrigated clay loam were increased to a greater extent when NH_4NO_3 (160 lb N per acre) was applied at midseason or split between planting and midseason than when all the N was applied at planting. Petiole NO_3^-% in the later part of the season was well correlated with yields. A. H. CORNFIELD.

Effect of major element levels on potato yields. P. S. Benepal (*Am. Potato J.*, 1967, **44**, 187–194).—Yields of potatoes on sandy loams in Panjab increased with level of applied N up to 300 lb per acre. 75 lb P_2O_5 per acre increased yields slightly in one experiment, whilst 75 lb K_2O had no effect. Tuber size was increased to the greatest extent by the heaviest level of N. A. H. CORNFIELD.

Effect of stones on potato yields, soil temperature and moisture. G. R. Saini and A. A. MacLean (*Am. Potato J.*, 1967, **44**, 209–213).—Yields of potatoes on a loam increased with the % of stones (2·5 cm dia.) up to 12% on a vol. basis in the upper 8 in. of soil, but declined somewhat with 24% stones. Temp. and moisture content of the surface soil increased with the % of stones present. A. H. CORNFIELD.

Effect of physiological maturity and storage temperature on emergence and yield of potatoes. H. J. Murphy, F. E. Manzer, M. J. Goven and D. C. Merriam (*Am. Potato J.*, 1967, **44**, 227–231).—Variations in maturity of seed stock (90–138 days) had no effect on plant emergence or yields. Seed stored at 3·3° produced higher yields than did seed stored at 0° or 7·2°. A. H. CORNFIELD.

Effect of nitrogen fertilisation on yield, composition and quality of table beets, *Beta vulgaris*. S. Shannon, R. F. Becker and M. C. Bourne (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 201–208).—Application of NH_4NO_3 (70–300 lb N per acre) to seven soils (ranging in texture from fine sandy loam to silt loam) increased total root yields and size of roots in proportion to level of N applied. The 150 lb N rate gave the most favourable economic return. Increasing N rate decreased sol. solids and red pigment content of the roots and increased total N and glutamine. No off-flavours were detected in the processed sliced beets due to any level of applied N. A. H. CORNFIELD.

Pasture plants for Northern Tanzania. IV. Legumes, grasses and grass-legume mixtures. Z. Naveh and G. D. Anderson (*E. Afr. agric. For. J.*, 1967, **32**, 282–304).—Results of many trials with a large number of varieties and strains of grasses and legumes in pure and mixed stands are reported with respect to dry matter and crude protein production. A. H. CORNFIELD.

Comparison between strains of *Eragrostis curvula* in South Africa. J. H. Leigh (*Expl. Agric.*, 1967, **3**, 327–335).—Herbage yields and the % and uptake of N, P, and K were studied in 20 strains of *Eragrostis curvula*. A. H. CORNFIELD.

Effects of fertilisers on grazed and cut elephant grass leys at Kawanda Research Station, Uganda. D. Stephens (*E. Afr. agric. For. J.*, 1967, **32**, 383–392).—Where elephant grass, *Pennisetum purpureum*, was cut and removed each year for 3 years K and Mg, in addition to N and P, applications were necessary to maintain good yields after the first year. Where the grass was grazed only N and P applications were required. On grazed grass the response to the higher rate of N (140 lb per acre) was particularly marked after the first year and its residual effects were large. A. H. CORNFIELD.

Effect of quackgrass, *Elytrigia repens*, on subsequent growth of sugar-beet. F. Plhák (*Pl. Soil*, 1967, **27**, 273–284).—Growth of sugar-beet following the growth and removal of quackgrass (*Q*) from the soil was inhibited when the soil was allowed to rest for a short period after *Q*, but temporarily stimulated when the soil was rested for a longer period. Available soil N was considerably decreased and available K somewhat decreased during growth of quackgrass. Fourteen days after removal of *Q* available N returned to the level of the control treatment, but available K hardly changed. Availability of P was not affected by *Q*. The depressing effect of *Q* on subsequent growth of sugar-beet is thought to be due to excretion of toxic substances from its underground parts affecting microflora and/or the succeeding crop. A. H. CORNFIELD.

Nitrogen fixing capacity of legumes in Nigeria. O. L. Oke (*Expl. Agric.*, 1967, **3**, 315–321).—The fixation of N_2 and the rate of transfer of N from nodules to the plant were studied for pigeon pea (*Cajanus cajan*), *Centrosema pubescens*, and *Styloxanthes gracilis* in pot tests. Fixation reached a max. of 0·0145, 0·0103, and 0·0046 g per plant per day respectively. The rate of transfer

of N from nodules to plant was constant over the whole 3 months of growth. In pigeon peas and *Centrosema* young plants were more efficient fixers of N_2 than were old plants, but the reverse was true in *Styloxanthes*. A. H. CORNFIELD.

Effect of growing a legume with grasses on grass nitrogen content and mineralisation of soil nitrogen. H. F. Birch and H. W. Dougall (*Pl. Soil*, 1967, **27**, 292–296).—Napier, Guatemala, and Guinea grasses grown in association with the legume *Desmodium uncinatum* were significantly higher in N% than when grown in pure stand in the first cut but not in later cuts. The presence of the legume, in comparison with the grass alone, considerably increased N% in the 0–0·5 in. soil layer and also increased mineralisable N in the next 3 in. soil layer. A. H. CORNFIELD.

Responses of lucerne to different soil moisture regimes. J. J. Landsberg (*Expl. Agric.*, 1967, **3**, 21–28).—Soil moisture content under lucerne in a granite sand receiving varying levels of application of irrigation water was studied. Both plant height and ground cover decreased with increasing moisture stress (decreasing irrigation). Relative turgidity was closely related to soil moisture content in the early morning, dry treatments showing more rapid recovery of turgor than those where water was kept at more adequate levels. Utilisation of radiant energy by the crop was affected by soil moisture and temp. A. H. CORNFIELD.

Irrigation frequencies for lucerne on shallow granite sand in low rainfall areas of Rhodesia. J. J. Landsberg (*Expl. Agric.*, 1967, **3**, 13–20).—Mature lucerne was irrigated at different intervals ranging from 0·4 to 1·0 × daily evaporation (E_0) as measured by a USWB Class A pan. Moisture balances were calculated for each treatment and when the accumulated sum of daily evaporation, multiplied by the appropriate factor, reached 1·2 in. (50% of the available moisture in the top 2 ft of soil) this amount of water was applied to the treatments concerned. Irrigation on the basis of 0·8 E_0 gave the highest yields over a year. During the rainy season yields from all treatments declined markedly, particularly those from the most frequently irrigated treatment. Although yields from the low-irrigation treatments were relatively poor during the dry season, the plants suffered no ill-effects and yielded well during the rains. A. H. CORNFIELD.

Russeting of Golden Delicious apples as related to soil application of sodium nitrate. D. E. Eggert and A. E. Mitchell (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 1–8).—Over 3 years annual application of $NaNO_3$ (4–8 lb per tree) increased the % of russeted fruit and leaf-Mg% and decreased leaf-K% and P%. Leaf-K and -P levels were negatively and leaf-Mg positively correlated with russeting. Leaf-N level was correlated with russeting in only 1 of 3 years. The $NaNO_3$ treatment increased the incidence of fruit shrinkage during the morning hours in the period shortly after bloom. A. H. CORNFIELD.

Effect of copper on yield and uptake of phosphorus and iron by citrus seedlings grown at various phosphorus levels. W. F. Spencer (*Soil Sci.*, 1966, **102**, 296–299).—In pot experiments with mandarin seedlings, the interaction between Cu and P is examined using four rates of each of Cu and P which were mixed with the soil and incubated for two months prior to planting. The soil contained ~98% of sand, 0·5% of clay and 1·0–1·5% of org. matter and had an exchange capacity of 2·5 mequiv./100 g. P was added as mono- or di-Ca phosphate (1 : 9), with Cu as $CuSO_4$. Toxic effects of high levels of Cu were suppressed somewhat by P and yields were increased. Excessive use of Cu lowered the uptake of P by the seedlings, diminished the top : root ratio and decreased the Fe content of leaves and roots. The P content of roots was lowered by Cu at all P levels tested. A. G. POLLARD.

Use of soil, water, and leaf analysis in growing oranges in Jordan. R. F. Allbrook (*Expl. Agric.*, 1967, **3**, 215–221).—Tentative standards for N, P, K, Na, Fe, and Mn in orange leaves for indicating nutrient status are presented. Conductivity values and anion and cation levels of soil and irrigation water are given in relation to possible toxic effects on orange tree growth. There was a good correlation between leaf and soil levels for K but poor correlation in the case of P. Leaf N values correlated well with amount of N applied to the soil. A. H. CORNFIELD.

Effect of time of harvest of Valencia oranges on leaf carbohydrate content and subsequent set of fruit. R. H. Hilgeman, J. A. Dunlap and G. C. Sharples (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 110–116).—As harvest date of mature Valencia oranges was advanced by monthly increments from March to July so fruit set of the following crop was reduced. Leaf total carbohydrate % decreased considerably from Feb. to early June and then increased somewhat to mid-July. The larger set of young fruit on trees harvested in March and April was associated with increased leaf carbohydrate in May. A. H. CORNFIELD.

Effect of boron nutrition on peach anatomy. A. R. Kamali and N. F. Childers (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 33–38).—The anatomy of the leaf, stem, trunk and fruit tissues of peach trees deficient in B or receiving excess B is described. External appearance of the fruit was similar where B was deficient or in excess, but parenchyma cells of the inner mesocarp of deficient fruit were corky with pronounced suberisation of the cell wall. A. H. CORNFIELD.

Tolerance of three varieties of olive to soil salinity in Israel. N. Bidner-Barhava and B. Ramati (*Expl. Agric.*, 1967, **3**, 295–305).—There were differences in tolerance to salinity between three varieties of olive. However, yields of fruit and oil from the most susceptible variety even on the saline soil were higher than those of the less susceptible varieties on the non-saline soil. A. H. CORNFIELD.

Field and laboratory studies of zinc fertilisation of pea beans and maize in 1965. F. Brinkerhoff, B. Ellis, J. Davis and J. Melton (*Q. Bull. Mich. St. Univ. agric. Exp. Stn*, 1966, **48**, 344–356).—Various inorg. carriers of Zn were tested using pea beans and maize as test crops. Zn-EDTA proved a useful Zn source, used in granular or powdered form; it could be mixed with fertilisers with little loss of efficiency. Zn clinker gave satisfactory results if treated with H_2SO_4; heavy applications were necessary if the acid pre-treatment was omitted. Zn frits were less efficient than was an equal amount of Zn as $ZnSO_4$. Inorg. Zn carriers were more effective if hand-mixed with fertiliser than if incorporated in the fertiliser granules. Residual effects of Zn applied 3 years previously were minimal. Maize yields were not increased by application of Zn. Use of P in excess of the amounts given at planting lowered leaf-Zn and in one of three localities depressed yields. A. G. POLLARD.

Effects of fertilisers and inoculation on yields of beans, *Phaseolus vulgaris*. D. Stephens (*E. Afr. agric. For. J.*, 1967, **32**, 411–417).—On a ferralitic loam (pH 5·1) bean yields were increased by application of superphosphate, CaO and $(NH_4)_2SO_4$, but not by urea, $CaSO_4$, Na_2MoO_4, or inoculation of the seed with N-fixing bacteria. Bean roots were weakly nodulated and the response to $(NH_4)_2SO_4$ was due to the extra effectiveness of its N over that of urea rather than to its SO_4^{2-} content. Leaf-P% was a useful guide to the P status of the plant, but leaf-N% or -S% were poorly correlated with yields. A. H. CORNFIELD.

Nature of boron stimulation to root initiation and development of beans. C. J. Weiser and L. T. Blaney (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 191–200).—Addition of H_3BO_3 to water stimulated earlier rooting from submerged hypocotyls and produced more and longer roots than did water alone. However, the number of root initials was not affected, indicating that B stimulated root growth and not root initiation. Sr^{2+}, Al^{3+}, and Ge^{4+}, with complexing properties similar to those of B, had no stimulatory effect on rooting. It is suggested that the oxidative root-stimulating mechanism may involve some phase of acid metabolism in the cuttings; possibly B stimulated movement of O-rich citric and isocitric acids from the leaves to the immersed hypocotyls which were O-poor. A. H. CORNFIELD.

Relation of calcium nutrition to hypocotyl necrosis of snap bean, *Phaseolus vulgaris*. S. Shannon, J. J. Natti and J. D. Atkins (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 180–190).—There were varietal differences in the susceptibility of snap bean to hypocotyl necrosis. The incidence of the disorder was inversely related to the Ca content of the seed. Ca deficiency was the main factor contributing to the disorder. In sand culture tests 10 mequiv. Ca^{2+} per l of solution eliminated the disorder; in this respect, Mg^{2+} was about half as effective as was Ca^{2+}. Even a normally resistant variety showed high incidence of the disorder when grown in nutrient deficient in Ca. A. H. CORNFIELD.

Effect of cultural conditions on yield and quality of carrots. G. A. Bradley, D. A. Smittle, A. A. Kattan and W. A. Sistrunk (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 223–234).—Carrot yields were increased by more frequent irrigation (every 7 days compared with every 10–14 days) only in the later harvests. The best colour ratings were obtained in carrots having high β- and low α-carotene contents. Colour and carotene contents were greatly affected by pre-harvest temp. The best colour and the highest β- to α-carotene ratios were obtained where soil and air temp. was in the region of 17°. A. H. CORNFIELD.

Effects of auxins on root initiation of stem tip cuttings from mature asparagus plants. D. C. Andreassen and J. Howard (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 158–162).—Rooting of stem tip cuttings from mature asparagus cuttings was initiated in culture solution containing naphthyl-1-acetic acid (5 ppm) or indole-butyric acid (10 ppm). Higher levels of either tended to delay rooting. Attempts to establish rooted cuttings outside the test-tube were not successful. A. H. CORNFIELD.

Correlations between coffee yields and soil analyses in Uganda. D. Stephens (*E. Afr. agric. For. J.*, 1967, **32**, 456–458).—Analysis of results from a number of sites over a number of years showed that only rarely were significant correlations obtained between coffee yields and soil total N, org. C, pH and extractable major elements. Even though N was the main element limiting yields in no case were yields significantly and positively correlated with org. C., total N, and C/N ratio. A. H. CORNFIELD.

Effects of nitrogen and magnesium fertilisers on coffee in Uganda. D. Stephens (*Expl. Agric.*, 1967, **3**, 191–203).—Experiments on soils ranging in texture from sand to clay loam showed that N was the main nutrient limiting yields of coffee. Application of Mg or S had little effect on yields. Application of N as $(NH_4)_2SO_4$ usually produced somewhat higher yields than did application as urea. Mulching had no effect on the responses to N fertilisers. The optimum time for application of N was in Feb. and Sept. A. H. CORNFIELD.

Response of peasant-grown Arabica coffee to cultural conditions on Mount Kilimanjaro, Tanzania. J. B. D. Robinson (*E. Afr. agric. For. J.*, 1967, **32**, 426–444).—Coffee yields per unit area from trees treated with $(NH_4)_2SO_4$ and Cu fungicides increased with altitude (3500–5500 ft) and closer spacing of trees. Yields increased to a small extent with increase in the density of interplanted bananas. There was no significant relation between yields and age of trees once they were in bearing, presence of interplanted yams or number of shade trees present in the coffee. A. H. CORNFIELD.

Chronic leaf fall in Arabica coffee in Tanzania. M. A. Hollies (*E. Afr. agric. For. J.*, 1967, **32**, 404–410).—Chronic leaf fall of apparently healthy leaves of Arabica coffee was prevented by spraying with Cu fungicides, but keeping the foliage dry also gave good leaf retention. There was no evidence that the action of Cu was physiological. Spraying seedlings with Cu fungicides did not increase the net assimilation rate, and good leaf retention due to spraying did not correspond with higher leaf chlorophyll content. Since fungicides other than Cu types were effective in leaf retention, chronic leaf fall is probably due to a pathogen rather than to any physiological cause. A. H. CORNFIELD.

Responses of mature tea to phosphate and potash in North-East India. A. R. Sen (*Expl. Agric.*, 1967, **3**, 55–62).—The effects of P, K, and P + K in the presence of varying levels of N on tea yields on a number of soil types are presented. A. H. CORNFIELD.

Salinity effects on growth of sugarcane. Lii-Jang Liu (*J. Agric. Univ. P. Rico*, 1967, **51**, 201–209).—There were significant differences in germination%, root dry wt. and stem growth between 10 varieties of sugarcane with respect to increasing soil salinity. The most resistant variety showed no damage when grown in soil with conductivity of 20 mmho per cm, whilst the least resistant variety showed damage when soil conductivity exceeded 5·4 mmho per cm. A. H. CORNFIELD.

Evaluation of sucrose–enzyme relationships in twelve Puerto Rico sugarcane varieties. A. G. Alexander (*J. Agric. Univ. P. Rico*, 1967, **51**, 29–38).—There were considerable variations in sucrose, fructose, amylase, invertase, tyrosinase, and peroxidase levels in the leaf and meristem of 12 varieties of sugarcane. Leaf phosphatase was particularly active in varieties producing low sugar yields. Invertase and, in particular, tyrosinase and peroxidase activity in the meristem were also high in the low sugar producers. The sugar-producing ability of test varieties can be assessed at a very early age by measuring enzyme activities. A. H. CORNFIELD.

Responses of sugarcane to supplementary irrigation on two soils in Natal. G. D. Thompson, J. M. Gosnell and P. J M de Robillard (*Expl. Agric.*, 1967, **3**, 223–238).—Yields of sugarcane on a sand and a clay increased with level of irrigation water applied. Even with the heaviest irrigation treatment the crop utilised water to a depth of 6 ft in the sand and 3 ft in the clay. Without irrigation the crop utilised water held at tension > 15 bars only in the surface foot of the sand, but in the top 3 ft of the clay. A. H. CORNFIELD.

Effects of major and trace elements and lime on cotton yields on Buganda clay loam. D. Stephens (*E. Afr. agric. For. J.*, 1967, **32**, 320–325).—Cotton yields on a ferralitic clay loam (pH 5·2) were increased by application of N, CaO and K, but not by Mn, B, Zn, Cu, Fe, and Mo. Responses were usually similar whether N was applied as $(NH_4)_2SO_4$ or $NaNO_3$, and a single application was as effective as a split application. The sixth opened leaf (without petiole) from the top of the main stem sampled after 'squaring' was the most suitable for indicating the nutritional status of the plant. A. H. CORNFIELD.

Effects of nitrogen fertilisation on cotton under boll weevil attack in North Carolina. W. J. Mistric, jun. (*J. econ. Ent.*, 1968, **61**, 282–283).—Increasing the application of N fertiliser from 35 to 105 lb/acre increased the no. of adult weevils, total squares, unpunctured squares, blooms, bolls and yields. Greater weevil activity did not negate increased fruiting. C. M. HARDWICK.

Effect of fertilisers on coconuts on the sandy soils of the Tanganyika coast. G. D. Anderson (*E. Afr. agric. For. J.*, 1967, **32**, 310–314).—A comparison of N, P, and K applied alone or in combination showed that the greatest increase in yields of husked nuts per tree occurred where N + K was applied. However, yields were significantly increased by all treatments. A. H. CORNFIELD.

Stimulation of latex flow in *Hevea brasiliensis*. P. D. Abraham and R. S. Tayler (*Expl. Agric.*, 1967, **3**, 1–12).—A review dealing with types of stimulants, formulations, methods of application, and mode of action. A. H. CORNFIELD.

Interrelation of potassium and chlorine supply on the burn of flue-cured tobacco. N. L. Pal, M. Bangarayya and Y. Ch. Narasimhamurthy (*Soil Sci.*, 1966, **102**, 346–352).—Sand-cultured tobacco plants were supplied with different levels of K^+ and Cl^-, all other elements in the nutrient being constant. The levels of K^+ and Cl^- affected the no. of curable leaves, wt. of cured leaves per plant or total bright leaf equivalent. High levels of K^+ increased leaf thickness; Cl^- had no effect. The Cl^- content of leaves was primarily controlled by the $[Cl^-]$ of the nutrient, but with increasing supply of K^+ the uptake of Cl^- diminished. The K^+ content of the leaves was related to the K^+ supply; Cl^- had little effect on K^+ uptake. Leaf-burn was increased by application of K^+ and decreased by that of Cl^-. An equation was established describing the relation between leaf-burn and the K^+ and Cl^- contents. (29 references.) A. G. POLLARD.

Introduction of the rain-forest species *Araucaria cunninghamii* (hoop pine) to a dry sclerophyll forest environment. B. N. Richards (*Pl. Soil* 1967, **27**, 201–216).—The major factor limiting establishment of hoop pine on the lateritic podzolic soils of coastal lowlands was N. Without added N there was virtually no growth, whilst 108 lb N per acre produced growth comparable with that occurring in rain-forest sites. When N deficiency was corrected a slight response resulted from P application. A. H. CORNFIELD.

Fertiliser and cultural requirements of tanier, *Xanthosoma* sp., on steep latosols. F. Abrūna-Rodríguez, E. G. Boneta García, J. Vicente-Chandler and S. Silva (*J. Agric. Univ. P. Rico*, 1967, **51**, 167–175).—Taniers grown under 50% shade provided by trees yielded only 33% as much as those grown in full sunlight. Yields were as high when plantings were made in undisturbed latosols as when pre-planting tillage was carried out. Prometryne was a satisfactory pre-emergence herbicide. Yields were similar with plant populations of 4840 to 14,520 per acre where rainfall was adequate, but increased with plant population where rainfall was limiting. Yields were consistently increased by application of 100 lb N per acre, but showed variable responses to P and K applications. $(NH_4)_2SO_4$, NH_4OH, NH_4NO_3, NH_4NO_3–$CaCO_3$, and urea were equally effective as sources of N, but $NaNO_3$ reduced yields seriously. A. H. CORNFIELD.

Pest Control

Some new nematocides and suggestions for their use. V. G. Perry and H. N. Miller (*Phytopathology*, 1967, **57**, 9).—Zinophos, Bay 25141 [*O,O*-diethyl *O*-*p*-(methylsulphinyl) phenyl phosphorothioate], diazinon, Mocap (*O*-ethyl *S,S*-dipropyl phosphorodithioate), NIA 10242 (2,3-dihydro-2,2-dimethyl 7-benzofuranyl *N*-methylcarbamate and UC 21149 [2-methyl-2-(methylthio)propionaldehyde-*O*-methylcarbamoyl oxime] were tested as nematocides. The three first-named materials were used as soil drenches, in granular form for direct application to soil, or as bare root dips for

control or eradication of *Meloidogyne* spp., *Radopholus similis*, *Pratylenchus coffeae* and other nematode parasites of *Philodendron* spp., *Sansevieria* spp., and *Gardenia jasminoides*. Bay 25141, NIA 10242 and UC 21149 were effective as soil drenches or granules or root dips for controlling *Belonolaimus longicaudatus*, *Hoplolaimus coronatus* on turf grasses. All chemicals named controlled *B. longicaudatus* and other nematodes on maize when granular forms were applied to the soil surface immediately before planting, and all were relatively non-phytotoxic. Max. reduction in no. of nematodes usually occurred 6–10 weeks after applications.
A. G. POLLARD.

Fungistatic properties of optical isomers of 2-aminobutane. J. W. Eckert and M. J. Kolbezen (*Phytopathology*, 1967, **57**, 98).—In neutral aq. solutions the hydrochlorides of enantiomers of 2-aminobutane (**I**), showed marked differences in fungicidal activity in preventing the germination of spores of *Penicillium digitatum*, *Phomopsis citri* and the mycelial growth of *P. digitatum*, *Monilinia fructicola* and *Glomerella cingulata*. It is concluded that control of *Penicillium* decay on orange by racemic (**I**) is largely due to the *d*(-) isomer. A. G. POLLARD.

Nature of the wide-spectrum antibiotic produced by pathogenic strains of *Pseudomonas syringae* and its rôle in the bacterial canker disease of *Prunus persicae*. S. L. Sinden and J. E. DeVay (*Phytopathology*, 1967, **57**, 102).—The isolation and purification of the antibiotic from pathogenic isolates of *P. syringae* is described. Two-dimensional chromatography showed nine ninhydrin-positive spots of which eight corresponded with known amino-acids. The purified polypeptide was highly phytotoxic. Data suggest that the antibiotic and the phytotoxin are the same compound.
A. G. POLLARD.

Anisic acid esters as knockdown agents. H. Beckman and M. Fernandez (*J. econ. Ent.*, 1967, **61**, 311).—After 15 to 20 min. contact with methyl anisate the knockdown of *Drosophila melanogaster* lasted 3 h. C. M. HARDWICK.

Insecticide hormoligosis. T. D. Luckey (*J. econ. Ent.*, 1968, **61**, 7–12).—Day old *Acheta domesticus* were reared under sub-optimal conditions and fed 0·1–3333 ppm of 14 insecticides. The effect on their growth rate is described. At $\sim\frac{1}{4}$ of the lethal dose, insecticides stimulated growth. Possible mechanisms are suggested. (24 references.) C. M. HARDWICK.

Preliminary evaluation of soil insecticides for sugar-beet root maggot control. W. E. Peay, C. E. Stanger and A. A. Swenson (*J. econ. Ent.*, 1968, **61**, 19–21).—Of 11 compounds applied as granules 1 in. below the soil surface, Bayer 37289 (*O*-ethyl *O*-2,4,5-trichlorophenyl ethylphosphonothioate) and Stauffer N-2790 (*O*-ethyl *S*-phenyl ethylphosphonodithioate) reduced beet loss by 81% and no. of *Tetanops myopaeformis* by >80%, and increased yield. Results varied with soil type. C. M. HARDWICK.

Differences in fungistasis in some Saskatchewan soils, with special reference to *Cochliobolus sativus*. S. H. F. China (*Phytopathology*, 1967, **57**, 224–226).—Eleven soils examined were divided into four groups on the basis of a soil dilution test. The soils in each group were similar in type and in org. matter content. Fungistasis based on germination of spores by the buried slide technique decreased in the order peat > loam > clay > sand. Nine isolates of *C. sativus* used in these tests were divided into four groups according to similarity of response to fungistasis. These responses are regarded as due to the inherent germinability of the spores and, thus, as genetic characteristics. A. G. POLLARD.

Effect of soil temperature and moisture on the survival of *Phytophthora parasitica* and *Pythium aphanidermatum*. E. E. Trujillo and M. Marcley (*Phytopathology*, 1967, **57**, 9).—*P. parasitica* survives in soil as chlamydospores, which are sensitive to changes in temp. and moisture levels; at <10° and >35° or with moisture <10% survival is markedly reduced. At 5° the organism survives for <2 days and below 0° for a few min. only, whereas in moist soil at 16–34° it survives for long periods. The thermal end-point is 47° for 10 min. in soil at 25% moisture content. *P. aphanidermatum* survives under a wider range of conditions, e.g., in dry soil for many years and in freezing soil for many months; temp. >50° are lethal to oospores in moist soils and the death point is <10 min. at 55°. A. G. POLLARD.

Soil fumigation by injection and irrigation for the control of nematodes on hops. A. R. Maggenti and W. H. Hart (*Pl. Dis. Reptr*, 1967, **51**, 794–798).—Yields of hops over 2 years were increased to the greatest extent by injection fumigation with 1,3-dichloropropene (60 gal per acre) before planting in the first year, followed a year later by 1,2-dibromo-3-chloropropane (2·5 gal per acre) in the first irrigation. A. H. CORNFIELD.

Movement and persistence of methane-arsonates in soil. R. Dickens and A. E. Hiltbold (*Weeds*, 1967, **15**, 299–304).—Adsorbtion of methane-arsonate (**I**) by kaolinite and vermiculite increased with concn. of Na_2-**I** in the equilibrium solution. Kaolinite and limonite adsorbed much more **I** than did vermiculite and montmorillonite. Adsorption of **I** by soils increased with their clay content. There was little downward movement of surface-applied **I** in a clay loam under the influence of leaching, but downward movement was fairly rapid in a sandy loam. Oxidation of the methyl-C of **I** was associated with oxidation of soil org. matter in soil. In a loamy sand, but not in three soils of heavier texture, increasing decomposition of **I** relative to that of soil org. matter occurred with time of incubation, indicating microbiological adaptation to **I**. Decomposition of plant material added to the soil enhanced oxidation of **I**. A. H. CORNFIELD.

Evaluation of chemicals for control of *Verticillium* wilt in a subtropical calcareous soil. C. W. Averre III and J. W. Strobel (*Phytopathology*, 1967, **57**, 7).—Comparative tests with 27 fungicides for controlling *Verticillium* wilt are recorded. Okra was used as the test plant. Confinement of the fungicides to the soil mass was achieved by a surface seal of water or black polythene mulch, the chemicals being applied by injection 4–6 in. below the soil surface, or in water as drenches (3000 gal/acre). Fumigant-type nematocides, org. P. compounds and most fungicides were not effective when used as drenches. In early treatments, Larvacide, Vorlex and Telone-PBC gave promising results. A. G. POLLARD.

Inhibition of nitrifying chemolithotropic bacteria by several insecticides. A. L. Garretson and C. L. San Clemente (*J. econ. Ent.*, 1968, **61**, 285–288).—*Nitrobacter agilis* was sensitive to chlorinated hydrocarbons at 1 μg/ml. TDE and aldrin caused complete inhibition over a 14 day period, while lindane only delayed NO_3^- production. Parathion was as toxic as aldrin while malathion and Baygon had less effect. *Nitrosomonas europaea* was 100× more sensitive to lindane, malathion and Baygon. C. M. HARDWICK.

Effects of aldrin and DDT on soil fauna. C. A. Edwards, E. B. Dennis and D. W. Empson (*Ann. appl. Biol.*, 1967, **60**, 11–22).—Application of 1·25% aldrin dust (3 cwt per acre) or 2% DDT dust (2 cwt) did not affect the numbers of Lumbricidae, Enchytraeidae, or Nematoda in fallow soil over one year. The greatest effects on arthropods were those of DDT on mesostigmatid mites and of aldrin on entomobryid or isotomid Collembola and on Pauropoda. Most species of Collembola increased in DDT-treated plots, apparently because mesostigmatid mites were reduced. Both insecticides killed more pests than predators or beneficial animals. The effects of the insecticides were greatest during late summer and autumn. A. H. CORNFIELD.

Impact on colonies of honey bees of ultra-low volume (undiluted) malathion applied for control of grasshoppers. M. D. Levin, W. B. Forsyth, G. L. Fairbrother and F. B. Skinner (*J. econ. Ent.*, 1968, **61**, 58–64).—Mortality from aerial application of ultra-low vol. malathion was complete within 2 h in unprotected hives. Colonies covered with burlap lost fewer bees, decreasing with the length of time they were covered, up to seven days. A high residue of malathion was found in pollen when residues were high on lucerne.
C. M. HARDWICK.

Effects of organometallic compounds on Lepidoptera. D. A. Wolfenbarger, A. A. Guerra and W. L. Lowry (*J. econ. Ent.*, 1968, **61**, 78–81).—The mortality and wt. change of *Heliothis zea* and *H. virescens* when one Sn, five Pb or two germane compounds were applied as foliar sprays or topically to larvae, are given. In field tests, hydroxytriphenyltin reduced the larval wt. of *Trichoplusia ni* and *Heliothis* spp. and reduced the no. of damaged squares.
C. M. HARDWICK.

Parathion and methyl parathion residues on cabbage and Southernpeas. C. E. Hoelscher, D. A. Wolfenbarger and N. E. Foster (*J. econ. Ent.*, 1968, **61**, 56–58).—Four applications of insecticide were given and the residues were analysed by gas chromatography, 14 days after the last application. Residues on cabbage were <1 ppm. Residues of methyl parathion were less than those for ethyl parathion at 4 and 7 days. Residues of both forms of parathion were more than the tolerance level of 1 ppm in peas and hulls, 5 days after application to Southernpeas.
C. M. HARDWICK.

Residues in spinach grown in disulfoton- and phorate-treated soil. R. E. Menzer and L. P. Ditman (*J. econ. Ent.*, 1968, **61**, 225–229).—Granular disulfoton and phorate were applied to spinach as pre-

planting and post-planting applications for control of *Myzus persicae*. Residues in the spinach were very persistent and consisted of parent compounds, sulfoxides, and sulfones, and the sulfoxides of the oxygen analogues. Winter applications were more persistent than summer applications, giving higher residues at harvest maturity. Analysis for total P and characterisation of residues by TLC are described. (18 references.)
C. M. HARDWICK.

Analysis of spray deposits on cards dyed with Sudan Black BR: their preparation and use. F. E. Skoog and F. T. Cowan (*J. econ. Ent.*, 1968, **61**, 40–43).—The prep. of Kromekote paper dyed with Sudan Black BR is described. This gave good definition of drops of malathion down to 15 μ in dia. Sunlight and i.r. heat lamps increased the contrast. A table is given of the relationship of drop size to spot size. C. M. HARDWICK.

Insect sex attractants. VIII. Structure-activity relationships in sex attractant for male cabbage loopers. M. Jacobson, H. H. Toba, J. De Bolt and A. N. Kishaba (*J. econ. Ent.*, 1968, **61**, 84–85).—Laboratory and field assay showed that the synthetic attractant *cis*-7-decen-1-ol acetate was more attractive to male *Trichoplusia ni* than the parent alcohol *cis*-7-decen-1-ol and the acetates of 7-octen-1-ol, 7-octyn-1-ol, *cis*- and *trans*-dodecen-1-ol, and 1-dodecanol. C. M. HARDWICK.

Effects of antibiotics and other compounds on fecundity and mortality of the two-spotted mite. F. H. Harries (*J. econ. Ent.*, 1968, **61**, 12–14).—Actinomycin D inhibited egg laying during the first 3 days of treatment, but was not significantly toxic to *Tetranychus urticae* for 7 days. Several other antibiotics exhibited similar characteristics. Dyes had no effect on mites.
C. M. HARDWICK.

Sex pheromone of the cotton leafworm. R. S. Berger (*J. econ. Ent.*, 1968, **61**, 326–327).—The compound was extracted from the abdomen of 4-day old *Alabama argillacea*. Its i.r. absorption spectrum and gas chromatograms suggest that it is a hydrocarbon with $\sim$ 20 carbon atoms. C. M. HARDWICK.

Sterilisation of codling moth by aerosol treatment with Tepa. D. O. Hathaway, B. A. Butt and L. V. Lydin (*J. econ. Ent.*, 1968, **61**, 322–323).—An apparatus is illustrated for delivering aerosol solutions for insecticidal treatment; 4000 moths could be treated per hour. 20% Tepa for 20 min. produced a high degree of sterility in *Carpocapsa pomonella* without affecting their mating ability. C. M. HARDWICK.

Suppression of *Drosophila melanogaster* in tomato field plots by release of flies sterilised by Apholate. H. C. Mason, T. J. Henneberry, F. F. Smith and W. L. McGovern (*J. econ. Ent.*, 1968, **61**, 166–170).—Flies were sterilised with 1% aq. Apholate and released at about 20–25 sterile males to 1 native male, during 1961–1962. Reductions of 86 and 44% in adults developing from eggs laid by trapped native females from two different areas were found. In field experiments there was a fly reduction of $\sim$80% for 7 weeks.
C. M. HARDWICK.

Sexual sterilisation of screw-worm flies by orally administered 1-[bis(1-aziridinyl)phosphinyl]-3-(3,4-dichlorophenyl) urea: effects of feeding times and concentrations of vehicle. M. M. Crystal (*J. econ. Ent.*, 1968, **61**, 140–142).—The chemosterilant was administered in a sugar syrup. It was not toxic. Degree of sterility rose as feeding time of males increased from 1 h reaching 95% at 16 h. There was a complex relationship between the degree of saturation of sugar and egg hatchability, the latter at 16 h being 17% (saturated solution) and 51% (0·1-saturated solution). C. M. HARDWICK.

Sexual sterilisation of screw-worm flies by *N,N*-tetramethylenebis (1-aziridinecarboxamide): influence of route of administration. M. M. Crystal (*J. econ. Ent.*, 1968, **61**, 134–139).—*Cochliomyia hominivorax* could not be sterilised by incorporation of chemosterilant in the larval medium or by immersion of prepupae. Adult flies treated topically, orally, by tarsal contact with a film, by injection or (as pupae) by immersion were sterilised. Topically sterilised males were more competitive than injected or contact-treated flies. Oral administration caused 50% toxicity. (26 references.) C. M. HARDWICK.

Histological studies of testes in rats treated with certain insect chemosterilants. B. D. Pate and R. L. Hays (*J. econ. Ent.*, 1968, **61**, 32–34).—Hydroxyurea, (I), triphenyltin acetate, (II), and triphenyltin chloride, (III), were administered to rats for 19 days. I produced no noticeable effects. II caused loss in body wt. and small testes showing degenerative changes; there was complete sterility. III produced similar but less marked changes; sterility was 60–70%. C. M. HARDWICK.

Pelletised lures for trapping the Mexican fruit fly. F. Lopez, L. M. Spishakoff and O. H. Becerril (*J. econ. Ent.*, 1968, **61**, 316–317).—Lures reconstituted from pellets attracted 10% fewer flies than did ordinary liquid bait. Pellets could be stored for 9 months with no loss of efficiency. A pelletting machine greatly reduced costs. C. M. HARDWICK.

Gas chromatographic analysis of insect attractant Cue-lure and related compounds. R. E. Doolittle and M. Beroza (*J. econ. Ent.*, 1968, **61**, 21–23).—A method for quant. analysis (to within 2%) of 4-(*p*-hydroxyphenyl)-2-butanone acetate is described. Possible impurities are discussed. C. M. HARDWICK.

Pulsed ultrasonic sound for control of oviposition by cabbage looper moths. T. L. Payne and H. H. Shorey (*J. econ. Ent.*, 1968, **61**, 3–7).—In one experiment, oviposition by cabbage looper moths was reduced 41, 23 and 30% when exposed to 20, 30 and 40 kHz respectively. There was also a reduction of oviposition (up to 66%) with increased sound intensity (70–90 db).
C. M. HARDWICK.

Respiration of confused flour beetle adults in carbon dioxide or nitrogen and after sublethal fumigation. S. D. Carlson (*J. econ. Ent.*, 1968, **61**, 125–131).—Exposure of adult *Tribolium confusum* to CO_2 or N_2 for 30 min. reduced O_2 consumption to a low level, at which it remained for 1·5 h. Afterwards O_2 consumption rose to a peak in 0·5–1 h. N_2 exposure also reduced CO_2 production. Fumigation with $CCl_4 : CS_2$ after preconditioning caused severe respiratory depression. Possible explanations are discussed. (15 references.) C. M. HARDWICK.

Respiration of the confused flour beetle in five atmospheres of varying carbon dioxide to ozygen ratios. S. D. Carlson (*J. econ. Ent.*, 1968, **61**, 94–96).—Using gas chromatography, the respiratory exchange of *Tribolium confusum* was determined at $CO_2 : O_2$ varying from 1 : 0·74 to 1 : 717. Low concn. of O_2 stimulated O_2 uptake. Concn. of CO_2 from 15–450 times >0·03% resulted in an output of CO_2 from 28–36% less than normal.
C. M. HARDWICK.

Laboratory tests of ultra-low-volume [ULV] and conventional low-volume [CLV] sprays for controlling bollworm and tobacco budworm. S. J. Nemic, P. L. Adkisson and H. W. Dorough (*J. econ. Ent.*, 1968, **61**, 209–213).—There was no difference in initial toxicity between ULV and CLV sprays. Residues from ULV sprays of methyl parathion were more toxic to *Heliothis zea* and *H. virescens* after 2 or 3 days than were those of CLV. This is attributable to a 4-fold deposit from the same dosage. Higher deposits were also found with Azodrin but both techniques were highly effective. (12 references.) C. M. HARDWICK.

Pick-up and penetration of ultra-low-volume [ULV] and emulsifiable concentrate [EC] malathion formulations by tobacco budworm larvae. T. M. Awad and S. B. Vinson (*J. econ. Ent.*, 1968, **61**, 242–245).—Third-instar *Heliothis virescens* picked up more malathion from leaves treated with ULV spray than from those given EC spray. When applied topically to larvae, 84% was recovered from ULV treatments after 24 h but that from EC treatment was about half this. A greater amount reached the site of toxicity in ULV treatments whereas much of the malathion from EC treatments was lost by evaporation or absorption by the cuticle.
C. M. HARDWICK.

Disease resistance in coffee berries. D. Hocking (*E. Afr. agric. For. J.*, 1967, **32**, 365–374).—A study was made of the possible defence mechanisms operative in the resistance of coffee berries to 'coffee berry disease'. A. H. CORNFIELD.

Factors in the resistance of apple to *Botryospaeria ribis*. J. Kuć, E. B. Williams, M. A. Maconkin, J. Ginzel, A. F. Ross and L. J. Freedman (*Phytopathology*, 1967, **57**, 38–42).—Golden Delicious apples became susceptible to *B. ribis* at about the mid-growth period. They were resistant to earlier inoculations. No fungitoxic compounds were detected in resistant apples. The onset of susceptibility occurred during a 1–2 week period. *B. ribis* grew on resistant apple tissue after the cells were ruptured. Oxidation products of the tissue did not inhibit the growth of the fungus. Oxidation products of phenolic substances inhibited pectolytic enzymes in culture filtrates from *B. ribis* to extents which declined as the apples matured. The inhibition was prevented but not reversed by chemicals which inhibit the browning of apple tissue (phenyl thiourea, $NaHSO_3$). (14 references.) A. G. POLLARD.

Spectrophotometric determination of 6-methoxy-2-benzoxazolinone [MBOA], an indicator of resistance to European maize borer in *Zea*

***mays*.** M. C. Bowman, M. Beroza and J. A. Klun (*J. econ. Ent.*, 1968, **61**, 120–123).—Extraction and spectrophotofluorometric measurement of 6-MBOA is described. It is a good indicator of resistance to first-brood larvae of *Ostrinia nubilalis*. Extracts contained a small amount of benzoxazolinone but this did not interfere with 6-MBOA analysis. Gas chromatography was not reliable for quant. analysis. C. M. HARDWICK.

Peroxidase activity and *Phytophthora* resistance in different organs of the potato plant. H. Fehrmann and A. E. Dimond (*Phytopathology*, 1967, **57**, 69–72).—A positive correlation is established between peroxidase activity in root, leaves, tuber peelings and pulp, and resistance to *P. infestans*. No such correlation was apparent between the chlorogenic acid content and resistance. Some correlation is indicated between polyphenol oxidase activity and tissue-resistance. A. G. POLLARD.

Development of resistance by the tobacco budworm to endrin and carbaryl. P. L. Adkisson (*J. econ. Ent.*, 1968, **61**, 37–40).—The resistance of 3rd and 4th instar *Heliothis virescens* to endrin and carbaryl by topical application was followed for 2 years. The high levels suggest cross-resistance. C. M. HARDWICK.

Migration of grasshoppers from plots sprayed with dieldrin in a Saskatchewan wheat field. R. L. Edwards (*J. econ. Ent.*, 1968, **61**, 106–109).—A fluorescent dye in aq. solution was applied, followed by another dye mixed with dieldrin. There was a reduction of grasshoppers in the sprayed plots over 4 days while the no. increased in the control plot. Many had migrated from the sprayed area. The significance of these results is discussed. C. M. HARDWICK.

Minimum number of insecticide applications for control of European maize borer on sweet maize in Quebec. M. Hudon (*J. econ. Ent.*, 1968, **61**, 75–78).—The first application of DDT or carbaryl was given 5 days after the beginning of egg laying and then weekly for up to 3 weeks. Over a 3 year period, three applications were most satisfactory for an early variety. For a late variety, one application, 15 days after the start of egg laying was satisfactory. There was little relationship between no. of applications and % of marketable ears. (16 references.) C. M. HARDWICK.

Effects of lindane (γ-benzene hexachloride) and carbaryl (1-naphthyl *N*-methyl carbamate) on rice yields. S. K. de Datta, R. S. Ayyangar and J. T. Magbanua (*Expl. Agric.*, 1967, **3**, 239–247).—In two crop seasons the highest rice grain yields were obtained by application of lindane (8 kg per ha) in three applications and weekly sprays of carbaryl (39 kg per ha over the season). In another season the highest yields were obtained where carbaryl (20 kg per ha) was incorporated in the soil and no lindane was applied. The carbaryl treatments reduced the numbers of stem borers and leafhoppers and the extent of virus infection. A. H. CORNFIELD.

Phytotoxicity of triphenyltin acetate to rice seed. D. Hocking and P. J. White (*E. Afr. agric. For. J.*, 1967, **32**, 380–382).—The presence of 5 ppm triphenyltin acetate (**I**) in the water completely inhibited germination of rice seeds, whilst 0·25 ppm produced stunted seedlings. The inhibitory effect of **I** was not reduced by 7 days exposure of the solution to sunlight. Reduction in phytotoxicity with time in soil is probably due to inactivation by adsorption on soil particles. A. H. CORNFIELD.

Interaction of propanil with insecticides applied as seed treatments on rice. C. C. Bowling and W. T. Flinchum (*J. econ. Ent.*, 1968, **61**, 67–69).—Propanil [*N*-(3,4-dichlorophenyl)propionamide] did not interact with chlorinated hydrocarbon when applied as a seed treatment. All carbamate insecticides except Matacil WP interacted to give leaf burn, as did three of seven phosphate insecticides. C. M. HARDWICK.

Growth, fruiting and storage response of five cultivars of bearing apple trees to *N*-dimethylamino-succinamic acid (Alar). D. V. Fisher and N. E. Looney (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 9–19).—Spraying apple trees with Alar (500–2000 ppm) once or twice during the month following full bloom resulted in reduced shoot and fruit growth in proportion to the concn. of Alar applied. Some russeting of Golden Delicious occurred. Fruits from treated trees were still firmly attached 3–4 weeks after normal harvest and these were still firmer than control fruit harvested at the normal time. Although treated fruits were firmer at harvest date, flesh colour, sol. solids %, and acid content indicated that they were as mature as controls. Sol. solids and titrable acidity were increased in some and decreased in other varieties and these differences persisted through the storage period. A. H. CORNFIELD.

Antifungal phenolic acids in apple fruits after infection with *Sclerotinia fructigena*. C. H. Fawcett and D. M. Spencer (*Ann. appl. Biol.*, 1967, **60**, 87–96).—Six phenolic compounds were isolated in the crude state from the antifungal materials produced in Edward VII apple fruits attacked by the brown rot organism, *S. fructigena*. Two of the compounds were identified as 4-hydroxybenzoic acid and 4-hydroxy-3-methoxybenzoic acid. They were shown to arise from the action of the pathogen on the juice of the fruit and not from the peel or the juice-free pulp. A. H. CORNFIELD.

An area control programme for pear psylla. E. C. Burts (*J. econ. Ent.*, 1968, **61**, 261–263).—All trees within 14,000 acres were sprayed with Perthane or related compounds as soon as *Psylla pyricola* began ovipositing. Aerial and ground sprays were used and in general good results were obtained. Growers were often able to cut out one or two summer sprays. (11 references.) C. M. HARDWICK.

Effect of storage temperature and sulphur dioxide concentration on decay and bleaching of table grapes. K. E. Nelson (*Phytopathology*, 1967, **57**, 100).—Data presented show the effects of temp. (31°, 35° and 39° F) of storage and of fumigation at weekly intervals with various [SO_2] (0·05, 0·1 and 0·2) on the spread of decay by *Botrytis cinerea* during storage periods from 39 to 120 days. A. G. POLLARD.

Chemotherapy of *Cytospora* canker and internal browning in Italian prune trees. A. W. Helton and W. J. Kochan (*Phytopathology*, 1967, **57**, 99).—In restricting the expansion of the canker the efficiency of 8-quinolinol benzoate, Phytoactin L-456 or cycloheximide thiosemicarbazone (**I**), each at 5000 ppm, increased in the order named. In concn. 50–3200 ppm, **I**, applied to 4-year old trees (in May and Aug.) prevented invasion by *Cytospora* and reduced internal browning; on 21-year old trees the decline in canker expansion reached up to 40% and that in internal browning up to 89%. Wound-paint prep. containing **I** or dimethyl-sulphoxide (**II**) prevented invasion of 4-year trees by *Cytospora*. Mixtures containing equal parts of **I** and **II** greatly reduced canker expansion in 21-year old trees. A. G. POLLARD.

Field evaluation of Temik against some insects and mites attacking citrus. J. C. Boling and H. A. Dean (*J. econ. Ent.*, 1968, **61**, 313–315).—Increasing applications of Temik from 25 to 50 g per tree on 4-year old trees reduced the numbers of citrus mites, live chaff scales and brown soft scales present. C. M. HARDWICK.

Wax emulsion additives for control of *Penicillium* storage decay of lemons. P. R. Harding, jun. (*Pl. Dis. Reptr*, 1967, **51**, 781–784).—Of a number of chemicals applied to lemons as wax emulsion sprays before storage thiabendazole (500 ppm) and 2,6-dichloro-4-nitroaniline (5000 ppm) were the most effective in reducing *Penicillium* decay during storage for 3 weeks. A. H. CORNFIELD.

Hot water treatments for control of *Penicillium digitatum* green mould of Eureka lemons. L. G. Houck (*Phytopathology*, 1967, **57**, 99).—Treatment of lemons, 2–3 or 7–8 days after picking, with hot water for 5–10 min. at 125° F or for 1 min. at 130° F followed by storage at 58° F and 85–95% R.H., reduced or completely prevented losses by green mould. Higher temp. or longer immersion adversely affected the fruit. A. G. POLLARD.

Oxygen diffusion, water, and *Phytophthora cinnamoni* in root decay and nutrition of avocados. L. H. Stolzy, G. A. Zentmeyer, L. J. Klotz and C. K. Labanauskas (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 67–76).—Addition of *Phytophthora cinnamoni* to the soil resulted in significant root decay and changes in the mineral nutrient concn. of roots and shoots of avocado. Root decay occurred in water-saturated soils even in the absence of the fungus. Extent of root decay was determined mainly by low level of O_2 supply in the root zone. A. H. CORNFIELD.

Prevention of stem-end rot, due to *Diplodia natalensis*, of the fruit of mango, *Mangifera indica*. V. N. Pathak and D. N. Srivastava (*Pl. Dis. Reptr*, 1967, **51**, 744–746).—Unripe mango fruit placed in cellophane bags immediately after picking showed no stem-end rot on ripening, whilst fruit exposed in the orchard for 8 h after picking showed 9–24% infection. This was prevented by treating the end of the pedicels with a paint consisting of $CuCO_3$,4, Pb_3O_4,4, and linseed oil, 5 parts. A. H. CORNFIELD.

Systemic chemical therapeutants for control of bean rust. E. K. Vaughan and S. R. Siemer (*Phytopathology*, 1967, **57**, 103).—The systemic fungicides, F461 (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin-4,4-dioxide), (**I**), and D735 (2,3-dihydro-5-carboxanilido-6-methyl-1,4-oxathiin), (**II**), were effective against bean rust,

(*Uromyces phaseoli*, f. *phaseoli*). I was more active than II for seed treatment. Both compounds caused temporary chlorosis of the primary leaves. When used as sprays at concn. 400 ppm, I was the more effective. A. G. POLLARD.

Increased cytokinin activity of rust-infected bean and broad-bean leaves. Z. Kirali, M. El Hammady and B. I. Pozár (*Phytopathology*, 1967, **57**, 93–94).—Following infection of the beans with *Uromyces phaseoli*, the cytokinin activity in the leaves increased. Methods of detecting the infection are examined. A. G. POLLARD.

Effect of timing of insecticide applications on infestation by *Cavariella aegopodii* Scop. on carrots. J. A. Dunn and D. P. H. Kempton (*Ann. appl. Biol.*, 1967, **60**, 33–42).—A single spray application of either demeton-methyl or menazon towards the end of the immigration period gave a similar overall control of aphid numbers on carrots to that given by disulfoton granules applied at sowing time. Minimal colonisation was obtained by two spray applications spanning the immigration peak, with 2 weeks between the demeton-methyl sprays and 3 weeks between those of menazon. The best control was obtained when the first application was made 7–10 days after the beginning of immigration. A. H. CORNFIELD.

Effect of level of nitrogen application on incidence of *Verticillium* wilt in hops. G. W. F. Sewell and J. F. Wilson (*Ann. appl. Biol.*, 1967, **59**, 265–273).—The incidence of wilt in hops increased with level of application of N [70–210 lb per acre as $(NH_4)_2SO_4$] at two sites over 3 or 5 years. There was a general decline in wilt incidence over the years. At one site wilt incidence was inversely related to soil temp. in the spring and early summer. In areas where wilt is a problem, N applications should be as low as possible commensurate with satisfactory yields. A. H. CORNFIELD.

Control of sweet potato weevil in Uganda. W. R. Ingram (*E. Afr. agric. For. J.*, 1967, **33**, 163–165).—Dipping the planting slips in DDT (2 lb per 8 gal water) reduced the % of damaged tubers, the degree of damage and the numbers of weevils (*Cylas formicarius*) but had no effects on yields. Spraying the plants at 10 and 16 weeks of age with DDT (2 lb per 24 gal) was ineffective. A. H. CORNFIELD.

Zinc deficiency in cotton induced by chloropicrin–methyl bromide soil fumigation to control *Verticillium* wilt. S. Wilhelm, A. George and W. Pendery (*Phytopathology*, 1967, **57**, 103).—Soil fumigation with a mixture (1 : 1, wt.) of chloropicrin and methyl bromide (225–375 lb/acre) greatly lowered the no. and kinds of microorganisms in the soil and normally improved plant growth. Cotton plants showed responses varying from favourable effects to severe stunting, chlorotic mottling and shortened internodes. Foliar sprays of 0·5% aq. $ZnSO_4$, alone or with other nutrients corrected these side-effects in a few days. Spraying with B, Cu, Fe, K, Mg, Mn, PO_4^{3-} or S had no effect. A. G. POLLARD.

Evaluation of seed-protectant chemicals against damping-off by *Fusarium* sp. and *Rhizoctonia solani* in cotton. S. L. Dongo and E. R. French (*Phytopathology*, 1967, **57**, 7).—The increasing incidence of damping-off of cotton seedlings in Peru in spite of increasing seed treatments, e.g. with Brassicol (**I**) and Rhizoctol (**II**) is examined. I and II controlled *Rhizoctonia* but not the *Fusarium*. Among other prep. tested a mixture of thiram and PCNB gave best results against both organisms without phytotoxic effects. A. G. POLLARD.

Effect of treating cotton seed with dieldrin, Abavit B, and aldrin on emergence of pink bollworm moths. A. Khalifa (*J. econ. Ent.*, 1968, **61**, 332–334).—Pupae of *Pectinophora gossypiella* were buried at varying depths in cotton seed. The % living moths emerging, decreased with depth. Very few living moths emerged from seed treated with Abavit or Abavit plus dieldrin. The average life of moths on untreated seed was 9·8 days and 1 h on treated seed. C. M. HARDWICK.

Outbreaks of two-spotted spider-mites and cotton aphids following pesticide treatment. I. Pest stimulation *vs.* natural enemy destruction as the cause of outbreaks. B. R. Bartlett (*J. econ. Ent.*, 1968, **61**, 297–303).—Of 59 pesticides tested on potted cotton plants in greenhouses against two-spotted mites and cotton aphids, 25 upset mite populations, 12 affected aphids and 12 upset both species. Toxicity ratings are given and max. periods of toxicity. Data are discussed in an attempt to explain causes of disturbance of populations as due to destruction of predators and/or direct stimulation of the pests. (31 references.) C. M. HARDWICK.

Evaluation in the greenhouse of a new insecticide for bollworm control. G. F. Ludvik, M. L. Randolph and W. A. Darlington (*J. econ. Ent.*, 1968, **61**, 304–306).—4′,5-dichloro-3-(4-chlorophenyl) salicylanilide was 5 to 7 times more toxic than Toxaphene-DDT to *Heliothis zea* larvae at LD_{50} and LD_{90} when caged on leaves of cotton plants. C. M. HARDWICK.

Control of pink bollworm larvae with soil-applied insecticide. T. F. Watson (*J. econ. Ent.*, 1968, **61**, 320–321).—Of seven insecticides applied to the soil, Aldrex, diazinon and parathion, produced sufficiently high mortalities of diapausing and non-diapausing *Pectinophora gossypiella* to be worth further testing. Wet soil was necessary for insecticidal activity. C. M. HARDWICK.

Constituents of the cotton bud. IX. Further studies on a polar boll weevil feeding stimulant complex. R. F. Struck, J. Frye, Y. F. Shealy, P. A. Hedin, A. C. Thompson and J. P. Minyard (*J. econ. Ent.*, 1968, **61**, 270–274).—Methods are described for the separation of a polar feeding stimulant by successive Soxhlet extractions of freeze-dried bud powder. One of the polar constituents was pptd. by Pb^{2+}. Gel permeation experiments suggest a mol. wt. of < 2000. C. M. HARDWICK.

Effect of 4′-(3,3-dimethyl-1-triazenyl) acetanilide [CL-24055] and other compounds on several lepidopterous pests of cotton. D. A. Wolfenbarger, W. L. Lowry, A. L. Scales and C. R. Parencia, jun. (*J. econ. Ent.*, 1968, **61**, 235–238).—In laboratory tests, CL-24055 sprays caused a reduction in wt. by limiting feeding in several species. The labial palps were shown to contain the sensory cells which react to cause this. Addition of sugar to the spray did not affect the results obtained. Nine compounds which do not affect feeding are listed. C. M. HARDWICK.

Preventative boll weevil control programme applied to a ten-square-mile area, [A] **Within a treated county.** W. J. Mistric, jun. and E. R. Mitchell. [B] **Within an untreated county.** W. J. Mistric, jun. and B. M. Covington (*J. econ. Ent.*, 1968, **61**, 179–186, 186–190).—[A] Azinophosmethyl-DDT, carbaryl and Toxaphene-DDT each applied at two dosages and two application intervals were compared. Relatively small differences in control of *Anthonomus grandis*, square production and yield were found. Boll weevil populations were reduced ~80% compared with controls.

[B] Early season application of Toxaphene-DDT sprays gave 95% control of overwintering weevils. Later migration from untreated areas increased the population of treated fields 856-fold. In spite of this treated fields had increased yields. C. M. HARDWICK.

Yield and response to fungicide and fertiliser on peasant-grown Arabica coffee on Mt. Kilimanjaro, Tanzania. III. Effects of treatments and seasons on yield. J. B. D. Robinson and R. G. Tapley (*E. Afr. agric. For. J.*, 1967, **33**, 123–130).—Standard Cu fungicide and N fertiliser treatment applied to Arabica coffee interplanted with bananas and shade trees increased crops yields significantly. The treatment effects were additive and there were no interactions. Yields and response to treatments varied with season. A. H. CORNFIELD.

Fungicides for Arabica coffee. I. Laboratory method for assessment against leaf rust, *Hemileia vastatrix*. D. Hocking and P. J. White. **II. Biological assessment of protective capacity against leaf rust.** D. Hocking. **III. Fungicides for Arabica coffee.** D. Hocking. **IV. Phytotoxicity of cycloheximide.** D. Hocking (*E. Afr. agric. For. J.*, 1967, **32**, 352–355, 356–358, 359–361, 363–364).—Cycloheximide (**I**) was as effective, at much lower deposit rates, as $CuOCl_2$ as a protectant against leaf rust infection. Several other materials were effective at only slightly higher deposit rates. I was the most effective material for curing leaf rust and gave virtually complete control with 0·0001 g active ingredient per sq. m. of deposit. I was phytoxic to leaves when the deposit exceeded 0·00025 g per sq. m. A. H. CORNFIELD.

Taint in Arabica coffee produced by spraying with lindane. D. N. McNutt (*E. Afr. agric. For. J.*, 1967, **32**, 347–351).—Spraying coffee trees in June and July with lindane (γ-$C_6H_6Cl_6$, 0·3–0·7 g active ingredient per tree) produced a greater taint in coffee from beans harvested late in the season than when harvested in the middle or early season. The lower rate of application of lindane resulted in less taint when applied in July than in June. A. H. CORNFIELD.

Fungicides for *Colletotrichum* leaf-spot and *Phytophthora* black pod rot of cacao. A. G. Newhall and F. Diaz (*Phytopathology*, 1967, **57**, 9).—In tests of 16 fungicides against the pod rot, Bordeaux mixture and Cu_2O prep. were more effective (being more tenacious) than Cu oxychlorides, $Cu(OH)_2$ and all org. fungicides. Against the leaf-spot, Difolatan and Dithane M-45 were superior to Fermate and DuTer. The density of Cu deposits on leaves was measured by pressing the sprayed leaves against filter paper pretreated with acidified dithioxamide. A. G. POLLARD.

Field-plot tests with insecticides for control of *Chironomus atrella*. S. W. Hitchcock and J. F. Anderson (*J. econ. Ent.*, 1968, **61**, 16–19).—Tests for larval control were carried out in open ended drums placed in the mud of a cove. Malathion, Abate, naled and synergised pyrethrum were effective as granules applied to the surface, as they settled in the mud. Max. mortality was ~95%. Possible side effects are discussed. (25 references.)
C. M. HARDWICK.

Response of striped Lynx spider, *Oxyopes salticus* to two commonly used pesticides. K. R. Redmond and J. R. Brazzel (*J. econ. Ent.*, 1968, **61**, 327–328).—Toxaphene–DDT was effective when applied topically or as a residue to spiders from two locations. Spiders from one location were more difficult (3-fold) to kill with residues of methyl parathion and 7-fold more with topical applications of methyl parathion. C. M. HARDWICK.

Bioassay of insecticides for control of *Drosophila melanogaster*. P. Singh, B. Taylor and J. G. Rodriguez (*J. econ. Ent.*, 1968, **61**, 336–337).—Rearing methods are described. Seven insecticides were used to prepare dry films. Day-old flies were exposed for 4 h. Diazinon was the most toxic insecticide but fenthion gave the quickest knockdown. C. M. HARDWICK.

Ethylene oxide against the greater wax moth. T. Lehnert and H. Shimanuki (*J. econ. Ent.*, 1968, **61**, 317–318).—Ethylene oxide (18 mg/l) killed all stages of *Galleria mellonella* in 24 h. The length of time necessary to kill the various stages at 36 mg/l of ethylene oxide is given. C. M. HARDWICK.

Topical toxicity of several chlorinated hydrocarbon, organophosphorus and carbamate insecticides to *Lygus hesperus*. T. F. Leigh and C. E. Jackson (*J. econ. Ent.*, 1968, **61**, 328–330).—LD_{50} values for 34 compounds over a period of several years are given. The LD_{50} doses and the regression coeff. are recorded. Chlorinated hydrocarbons generally required higher dosages than did carbamates or org. P. insecticides. C. M. HARDWICK.

Hydrocyanic acid and other fumigants for control of larvae of *Plemeliella abietina* and *Megastigmus* sp. in imported spruce seed. H. H. Richardson and H. Roth (*J. econ. Ent.*, 1968, **61**, 214–216).—HCN, particularly under vac., was at least as effective as MeBr, CCl_4 and ethylene oxide against both species. In germination tests, five conifer species tolerated 32 mg of HCN under vac.
C. M. HARDWICK.

Lindane controls *Lyctus* powder-post beetles during air-drying of rough ash-lumber. V. K. Smith (*J. econ. Ent.*, 1968, **61**, 323–324).—The lowest concn. of lindane to protect timber from *Lyctus* beetles was 0·06%. An emulsion, 25% stronger, was necessary for control of ambrosia beetles. C. M. HARDWICK.

Fluoroglide as a barrier to contain non-flying insects in glass containers. R. H. Storch (*J. econ. Ent.*, 1968, **61**, 335–336).—Chemically pure Fluoroglide (fluorocarbon resin) when painted round the rim of a beaker prevented the escape of second-instar *Coccinella septempunctata* but not that of first-instar *Myzus persicae*. The bonding grade was not satisfactory. C. M. HARDWICK.

Detection of potato viruses X, M and S in dormant potato tubers by the bentonite flocculation test. R. P. Kahn, H. A. Scott, J. Bozicevich and M. M. Vincent (*Phytopathology*, 1967, **57**, 61–65).—Data obtained from single-eye extracts of a wide range of species by the bentonite flocculation test (**I**) were compared with those by the indexing and micro-precipitin (**II**) tests using foliage extracts. Viruses X, M and S were detected by both **I** and **II**. Virus Y was not detected by **I** in single-eye tests but both **I** and **II** gave positive tests in foliage extracts. A. G. POLLARD.

Effects of supplying *N*-6-benzyladenine (BA) to leaves and plants inoculated with viruses. H. S. Aldwinkle and I. W. Selman (*Ann. appl. Biol.*, 1967, **60**, 49–58).—Application of BA was as effective as kinetin in decreasing the numbers of local lesions in petunia leaf strips infected with tomato spotted wilt virus (TSWV). Benzimidazole, adenine and NH_4NO_3 were ineffective. Local lesions and infectivity of TSWV in detached leaves of *Nicotiana rustica* were decreased by supplying BA before and after inoculation. Both were decreased in attached leaves when BA was applied 9 days before inoculation. BA supplied to attached leaves after inoculation increased infectivity. Supplying BA to the lower leaves of tomato plants before inoculating with TSWV decreased infectivity of unsprayed, systemically infected tip leaves taken as inoculum; BA supplied after inoculation increased infectivity. BA decreased the number of local lesions caused by lucerne mosaic virus in excised leaves of *Phaseolus vulgaris*. A. H. CORNFIELD.

Phenolase activity in *Nicotiana glutinosa* infected with tobacco mosaic virus. V. T. John and M. Weintraub (*Phytopathology*, 1967, **57**, 154–158).—Tyrosine and various phenolic substrates were oxidised by commercial tyrosinase and crude mushroom extracts; extracts of healthy leaves of *N. glutinosa* were unable to oxidise tyrosine unless the leaves were infected with tobacco mosaic virus. After infection the leaf extracts oxidised tyrosine more rapidly than other phenolic substrates. A. G. POLLARD.

Host nutrition affects virus infection. R. E. Foster (*Phytopathology*, 1967, **57**, 98).—In *Chenopodium amaranticolor* sites of infection with cucumber mosaic virus were hypersensitive to the mineral nutrition of the host plant. Counts of local lesions showed that high-N, high-P, low-K, high-Ca and high-Mg feeding, each favoured infection. No interaction was apparent between P in buffer solution and P-nutrition level or between K in buffer solution and K-nutrition, thus suggesting that the action of the buffer was on the virus rather than on the host tissue.
A. G. POLLARD.

Influence of a sub-acute infection of polyhedrosis virus in cabbage looper on susceptibility to chemical insecticides. J. H. Girardeau, jun. and E. R. Mitchell (*J. econ. Ent.*, 1968, **61**, 312–313).—When the insects were fed with a low count of polyhedra virus there was no change in the amount of insecticide necessary. If the virus was allowed to develop in the larva for 24 h prior to insecticidal treatment, the amount of endrin was reduced 3-fold and endosulfan 5-fold. After 48 h the amount of trichlorfon could be reduced 4-fold. (11 references.) C. M. HARDWICK.

Environmental factors and susceptibility to herbicides. J. L. Hammerton (*Weeds*, 1967, **15**, 330–336).—A review (71 references) dealing with the effects of rainfall, humidity, temp., light, soil conditions, effects of crop and other factors on the susceptibility of weeds to herbicide applications. A. H. CORNFIELD.

Influence of weed competition on sugar beets. R. L. Zimdahl and S. N. Fertig (*Weeds*, 1967, **15**, 336–339).—At comparable weed densities broadleaf weeds reduced yields of sugar beet more than did annual grass weeds. Beet tops and root dia. were affected in a manner comparable with that of total yields. Weeds had no effect on beet stand or the total solids % or sucrose % of the roots.
A. H. CORNFIELD.

Influence of herbicides on root development of Bermuda grass, *Cynodon dactylon*. S. W. Bingham (*Weeds*, 1967, **15**, 363–365).—In field trials application of DCPA (dimethyl-2,3,5,6-tetrachloroterephthalate, 10 lb per acre), Bensulide [*N*-(2-mercaptoethyl)-benzenesulphonamide *S*-(*O*,*O*-diisopropylphosphorodithioate), 15 lb] and Terbutol (2,6-di-t-butyl-*p*-tolyl-methylcarbamate, 10 lb) applied in early April delayed normal rooting of Bermuda grass from stolon nodes for about 12 weeks. Established root systems were not appreciably altered by the treatments.
A. H. CORNFIELD.

Studies on pyrazon [5-amino-4-chloro-2-phenyl-3(2*H*)-pyridizanone] under controlled environmental conditions. R. Frank (*Weeds*, 1967, **15**, 355–358).—Between emergence and the cotyledon stage both sugar beets and weeds were susceptible to pyrazon and phytoxicity increased with temp. (10–30°). In the late cotyledon stage beets were resistant to pyrazon in aq. suspension, but partially susceptible to an oil–water suspension. In pre-emergence treatments 2–4 lb pyrazon per acre had little effect on beets, but 6 lb caused slight mortality. When applied post-emergence, 4–6 lb caused fair mortality unless applied after expansion of true leaves.
A. H. CORNFIELD.

Residues of two herbicides in water in irrigation canals. P. A. Frank, R. H. Hodgson and R. D. Comes (*Weeds*, 1967, **15**, 353–355).—Residues of fenac (2,3,6-trichlorophenylacetic acid) and dichlobenil (2,6-dichlorobenzonitrile) applied to drained canal beds for control of aquatic weeds were quickly washed away when the water was allowed to flow through the canal. Concn. of the two herbicides in the water reached low levels within 1 h of the start of water flow and were reduced to negligible levels 2 miles downstream. A. H. CORNFIELD.

Effect of dipyridyl and toluidine herbicides on growth response of *Sclerotium rolfsii* in soil. E. A. Curl, R. Rodríguez-Kabana and H. H. Funderburk (*Phytopathology*, 1967, **57**, 7).—Effects of paraquat (**I**) and of trifluralin (**II**) on *Scl. rolfsii* are examined in a sandy loam soil. Measurements of CO_2 evolution showed restriction of respiration by **I** in the concn. range, 12·5–1000 ppm and by **II** with increasing concn. in soil >6·25 ppm. Reduced respiration, at higher concn. of either herbicide, was associated with lowered assimilation of NO_3^- and lowered production of non-volatile acids by the fungus. A. G. POLLARD.

Darkness and the activity of diquat and paraquat on tomato, broad bean and sugar beet. R. C. Brian (*Ann. appl. Biol.*, 1967, **60**, 77–85).—A period of darkness after treatment increased the activity of diquat and paraquat on the three species. The extent of the increase was dependent on the time of day and year of treatment, on the light quality and intensity before spraying and on the duration of the darkness. A. H. CORNFIELD.

Effects of atrazine (2-chloro-4-ethylamino-6-isopropylamino-*s*-triazine) and hydroxy-atrazine (2-hydroxy-4-ethylamino-6-isopropylamino-*s*-triazine) on nitrogen metabolism of selected plant species. E. F. Eastin and D. E. Davis (*Weeds*, 1967, **15**, 306–309).—Tests in soils and nutrient solution showed that the application of atrazine (1 lb per acre) increased the N% in all eight species, whether resistant or susceptible. The treatment usually reduced plant wt. and total N uptake. The treatment had no effect on plant non-protein-N% and decreased NH_3-N% in resistant species, but increased both fractions in intermediate and susceptible species. Hydroxy-atrazine, a degradation product of atrazine, had no consistent effect on any of the N fractions determined or on total N% of the plants. A. H. CORNFIELD.

Simazine [2-chloro-4,6-bis(ethylamino)-*s*-triazine] and phosphorus interactions in red pine, *Pinus resinosa*, seedlings. P. S. Dhillon, W. R. Byrnes and C. Merritt (*Weeds*, 1967, **15**, 339–343).—Uptake of P by red pine seedling roots from a medium containing 0·0015 ppm ^{32}P was stimulated by the presence of 5–10 ppm but inhibited by 15–20 ppm, simazine in the medium. Over 96 h, 5–20 ppm simazine increased the translocation of P from roots to stem and needles. P uptake was reduced by 10 ppm simazine from nutrient containing more than 20 ppm P. P% in needles was significantly increased, whilst that of stems was not greatly affected by, simazine treatments or varying P concn. of the nutrient. A. H. CORNFIELD.

Protecting lucerne seedlings from damage by a triazine with activated charcoal. D. L. Linscott and R. D. Hagin (*Weeds*, 1967, **15**, 304–306).—A suspension of charcoal in water sprayed on the soil surface (50 lb C per acre) directly over rows of seeded lucerne protected the seedlings against damage by a pre-emergence broadcast application of 2-isopropylamino-4-(3-methoxypropylamino)-6-methylthio-*s*-triazine, 1·5 lb per acre. Higher doses of charcoal were necessary when higher doses of the weedicide were applied. A. H. CORNFIELD.

Susceptibility of almond trees to herbicides in relation to type of rootstock and scion. A. H. Lange and C. L. Elmore (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 56–60).—There were differences in the susceptibility of two scion varieties (Mission and Nepulus) to simazine (8 lb) and isocil (4 lb per acre). Susceptibility was greater on a shallow- than on a deep-rooting rootstock. A. H. CORNFIELD.

Chemical control of red sorrel in Kentucky bluegrass seed fields. C. L. Canode and W. C. Robocker (*Weeds*, 1967, **15**, 351–353).—Selective control of red sorrel, *Rumex acetosella*, in Kentucky bluegrass seed fields was obtained by autumn application of 2,3,6-TBA (2,3,6-trichlorobenzoic acid, 1–4 lb), endothal [7-oxabicyclo (2.2.1)-heptane-2,3-dicarboxylic acid, 0·5–2 lb], dicamba (2-methoxy-3,6-dichlorobenzoic acid, 0·25–1 lb), amiben (3-amino-2,5-dichlorobenzoic acid, 2–4 lb), and fenac (2,3,6-trichlorophenylacetic acid, 2–4 lb per acre). Kentucky bluegrass seed yields and germination were not significantly affected by application of any of the herbicides even though rates higher than necessary for red sorrel control were used. A. H. CORNFIELD.

Control of rush skeletonweed, *Chondrilla juncea*. R. Schirman and W. C. Robocker (*Weeds*, 1967, **15**, 310–312).—There was consistently good control of skeletonweed by autumn application of picloram (4-amino-3,5,6-trichloropicolinic acid, 2 lb) and dicamba (2-methoxy-3,6-dichlorobenzoic acid, 8 lb per acre). Splitting the applications between autumn and spring was not as effective. A. H. CORNFIELD.

Metabolism of labelled amitrole (3-amino-1,2,4-triazole) in plants. S. C. Fang, E. Fallin and S. Khanna (*Weeds*, 1967, **15**, 343–346).—The metabolic conversion of amitrole in leaves was exponential and could be expressed by an equation for a first-order reaction. The half-time of amitrole in sugar-beet, maize, and bean leaves was 18·7, 28·0, and 23·2 h respectively. The largest amounts of radioactivity from 5-^{14}C-labelled amitrole were recovered as alcohol-sol. metabolites of the leaves, and radioactivity was also found in nucleic acids, proteins and other plant constituents. A. H. CORNFIELD.

Control of woody and herbaceous vegetation with soil sterilants. R. W. Bovey, R. E. Meyer, F. S. Davis, M. G. Merkle and H. L. Morton (*Weeds*, 1967, **15**, 327–330).—Of a number of soil sterilant herbicides tested bromacil (5-bromo-3-s-butyl-6-methyluracil, 40–160 lb per acre), prometon [2-methoxy-4,6-bis(isopropylamino)-*s*-triazine, 40–160 lb] and picloram (4-amino-3,5,6-trichloropicolinic acid, 10–80 lb) were the most effective. Most herbicides controlled vegetation for 1 year or less. Bromacil controlled vegetation for approx. 2 years at most locations. A. H. CORNFIELD.

Influence of envionmental factors on the efficacy of pre-emergence diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea] applications. J. E Bowen (*Weeds*, 1967, **15**, 317–322).—In Hawaiian sugarcane fields there was a highly significant negative correlation between the extent of weed control and the lapse of time between harvest date and date of diuron application. This was the most important factor influencing the pre-emergence activity of diuron. Variations in rainfall, soil org. matter content, and average daily max. temp. exerted significant, but relatively unimportant, effects on extent of weed control. A multiple regression equation relating extent of weed control to the variables studied is presented. A. H. CORNFIELD.

Phytotoxicity of herbicides to deciduous fruit tree seedlings. A. H. Lange and J. C. Crane (*Proc. Am. Soc. hort. Sci.*, 1967, **90**, 47–55).—Sand culture studies on the effect of 15 herbicides applied to the roots of almond, peach, plum, cherry, pear and black walnut are reported. A. H. CORNFIELD.

Influence of pH on detoxication of herbicides in soil. F. T. Corbin and R. P. Upchurch (*Weeds*, 1967, **15**, 370–377).—The rate of detoxication of 11 added herbicides during aerobic incubation of highly org. soils over the pH range 4·3 to 7·5 was studied using growth rate of cucumber seedlings as a measure of reduction in phytotoxicity. Dicamba and 2,4-D showed max. detoxication at pH 5·3, dalapon and amitrole at pH 6·5, and vernolate at pH 7·5, whilst detoxication of diuron and amiben was not affected by soil pH. Picloram, trifluralin, isocil, and prometon showed no detectable detoxication even after 8 weeks incubation. A. H. CORNFIELD.

Brush control in spaghnum moss bogs. M. N. Dana (*Weeds*, 1967, **15**, 380–381).—Black chokeberry, *Aronia melanocarpa*, in spaghnum moss bogs was controlled by spray application in the spring of picloram (4-amino-3,5,6-trichloropicolinic acid, 1 lb per acre). Leatherleaf, *Chamaedaphne calyculata*, was controlled by the same rate of picloram applied in spring or autumn. Eight other herbicides tested were ineffective. A. H. CORNFIELD.

Compositions for sterilising insects and methods of insect control. Rohm and Haas Pharma G.m.b.H. (Inventor: H. F. Goonewardene) (B.P. 1,079,010, 1.6.66).—An insect population with a reduced capacity for propagation is produced by treating insect larvae, pupae or insects themselves (houseflies) with a methacrylamide $CH_2 : C(CH_3)CONHR$, in which R is a straight- or branched-chain or cyclic alkyl group of >8 C atoms, e.g., *N*-t-butylmethacrylamide (**I**). Houseflies treated with **I** absorbed onto milk powder laid only 44 eggs (15 flies) compared with 1074 eggs laid by 15 control flies. J. M. JACOBS.

α, β-Dithiols. Société Nationale des Petroles d'Aquitaine (B.P. 1,080,830, 22.12.65. Fr., 23.12.64).—Used instead of 1,2-glycols and as raw materials for synthesis of insecticides and pesticides, the title compounds have the general formula $R^1(R^2)C(SH)C(SH)R^3(R^4)$, wherein R^1, R^2, R^3 and R^4 are (identical or different) H or alkyl. They are obtained by condensing H_2S and an alkaline base in aq. alkaline solution saturated with H_2S, with the corresponding 2-one-1,3 oxathiolane at 0–10°, to give an alkaline double salt which is acidified to give the required thiol, e.g. ethane dithiol in 62% yield. S. D. HUGGINS.

Pesticidal compositions for exterminating insects. Bombrini-Parodi-Delfino S.p.A. (B.P. 1,082,285, 22.1.65. It., 25.1.64).—A composition for extermination of insects which are resistant to org. Cl-containing insecticides (**I**) comprises a mixture of **I** and 0·25–1 pt. of a compound of formula $NRR^1CO_2R^{IV}$ wherein R^{IV} is 2-SR^{II}-4-R^{III}pyrimidyl and R-R^{III} are alkyl of 1–4 C, e.g., 2-methylthio-4-methylpyrimid-6-yl dimethylcarbamate (which synergises the insecticidal action of DDT and gamma-BHC). F. R. BASFORD.

Fungicidal compositions for agricultural and horticultural purposes. Ihara Noyaku K. K. (B.P. 1,078,662, 4.8.64. Jap.,

30.8.63 and 5.2.64).—The compositions contain (*a*) one or more compounds of formula $(RO)_2P(X)SCH_2C_6H_5$, wherein R is 1–4 C alkyl, X is O or S and the phenyl ring may be further substituted by $(Y)_n$ (where Y is halogen or $-NO_2$ and *n* is 0–2; where $n = 2$, one Y may be halogen and the other $-NO_2$) and (*b*) a carrier (dust, wettable powder or dil. emulsified concentrate). E.g., 15% by wt. of *S*-(*p*-chlorobenzyl)*O*,*O*-diethylthiolophosphate is mixed with 80% by wt. of diatomaceous earth–kaolin mixture and 5% by wt. of a spreader to give a wettable powder for application to paddy field rice plants infected by *Piricularia oryzae*.
S. D. HUGGINS.

***N*,*N*′-Disubstituted bipyridylium salts.** Imperial Chemical Industries Ltd. (Inventors: J. E. Colchester and J. H. Entwise) (B.P. 1,077,366, 28.5.65).—*N*,*N*′-Disubstituted-4,4′-bipyridylium halides are obtained by reacting *N*,*N*′-disubstituted-4-4′-bipyridyl with a C compound containing a labile halogen atom adjacent to -CO, $-CCl_3$, $-CBr_3$, $-CI_3$ or F, Cl or Br atoms in an ether, ketone, hydrocarbon, halogenated hydrocarbon, a pyridine, amide or sulphoxide at 0–50°, the product being extracted with water. The products have herbicidal activity. S. D. HUGGINS.

1,1′-Alkylene-2,2′-bipyridylium halides. Imperial Chemical Industries Ltd. (Inventors: J. T. Braunholtz and C. Shepherd) (B.P. 1,078,846, 5.6.63).—The title compounds are obtained by reaction of 2,2′-bipyridyl in a solvent with a mixture of halogen and l-alkene, preferably Cl_2 and C_2H_4, in the presence of 10–20% by wt. of catalyst at 80–160°. The compounds, e.g., 1-1′-ethylene-2,2′-bipyridylium dichloride, have herbicidal activity.
S. D. HUGGINS.

Animal Husbandry

Commercial standards for animal proteins. Anon. (*Infciones Grasas aceit.*, 1966, **4**, 373–374).—Standards for meat, bone and poultry meal are compared. L. A. O'NEILL.

Feeding value of solvent-extracted camelina meal. Effect of levels fed, heat treatments and flavour supplements. G. O. Korsrud and J. M. Bell (*Can. J. Anim. Sci.*, 1967, **47**, 109–114).—The meal was fed to weanling mice in proportions 0–25% of the diet, with small amounts of licorice to modify the flavour. When added to the diet in amounts up to 10%, camelina meal (**I**) had no ill effects on the mice but larger proportions lowered feed intake and gain in wt. In another trial 20–22% of **I** was included in all the diets, comparison being made between the raw meal, dry-heated meal (110°, 2 h), autoclaved meal (15 min., 1·2 kg/sq. cm), steam-stripped meal (110°, 2 h) and a casein–methionine-supplemented control diet. Myrosinase sources were added in each case. Destruction of myrosinase by heating increased the feeding value of the camelina meal; steam stripping was more effective than dry heating or autoclaving. Camelina meal can be used in amounts >10% of the diet without depression of feeding value; in myrosinase-free diets, 20% of the meal produced growth approaching max. At higher levels growth depression was greater than could be attributed to volatile isothiocyanates. A. G. POLLARD.

Effects of various heat and moisture treatments on myrosinase activity and nutritive value of solvent-extracted crambe seedmeal. G. O. Korsrud and J. M. Bell (*Can. J. Anim. Sci.*, 1967, **47**, 101–107).—The meal from *Crambe abyssinica*, Hochst., uncooked, dry heated or steam stripped was fed to mice in amounts 0, 5 or 10% of the diet together with rapeseed (1% of the diet) as a source of myrosinase. The uncooked meal adversely affected feed intake and gain in wt. of the mice but heating by either method partially corrected this. No appreciable effect of the rapeseed was apparent. In another test the ground seeds of crambe, rape and *Camelina sativa* were compared as myrosinase sources at the rate of 1 : 4 parts of cooked crambe meal after allowing the mixture to react overnight with 30% moisture content. Some hydrolysis of thioglucosides occurred and the product markely diminished growth rates. Feeding active myrosinase depressed animal responses, apparently through *in vivo* hydrolysis of thioglucosides. All sources of the enzyme, used in this way, were effective.
A. G. POLLARD.

Comparison of Giant Pangola (*Digitaria valida*), Signal grass (*Brachiaria brizantha*), and common Pangola (*Digitaria decumbens*) as pasture crops in the mountain region of Puerto Rico. L. Rivera-Brenes, A. Rodríguez-Carbera and H. Cestero (*J. Agric. Univ. P. Rico*, 1967, **51**, 193–200).—There were no significant differences in total digestible nutrient yield per acre and carrying capacity of heifers over 613 days between Signal grass and common Pangola; both were significantly superior to Giant Pangolagrass.
A. H. CORNFIELD.

Nutritive value of *Themeda triandra* hay. B. Marshall (*E. Afr. agric. For. J.*, 1967, **32**, 375–379).—Three samples of *Themeda triandra* hays had digestible crude protein 0–0·82, starch equiv. 31–37, and total digestible nutrients 49–61 expressed as % of dry matter. Its palatability to steers was also very low. Unpalatability was probably due to the low protein content of the material.
A. H. CORNFIELD.

Estimation of digestibility from *in vitro* rumen fermentation data in forages of Puerto Rico. J. A. Arroyo-Aguilú (*J. Agric. Univ. P. Rico*, 1967, **51**, 133–139).—*In vitro* cellulose digestion values, using the artificial rumen technique, are presented for Merker grass (*Pennisetum purpureum*), Guinea grass (*Panicum maximum*), and Buffel grass (*Cenchrus ciliaris*) harvested at 49–55 and 63–69 days of age. *In vitro* cellulose digestion after 36 h gave the best estimates of forage digestibility in terms of digestible dry matter, digestible energy, and total digestible nutrients. Regression equations for predicting these values are presented. A. H. CORNFIELD.

A simple respiration chamber for carbon-14 studies with pigs. H. M. Cunningham (*Can. J. Anim. Sci.*, 1967, **47**, 139–141).—The chamber described (air vol., 2250 l) includes temp. control (21°), air circulators with air flow 38 l/min., a Max Planck meter with continuous sampling device and a CO_2-absorber (ethanolamine and ethylene glycol monomethyl ether). Pigs are placed in the chamber within 2 min. of receiving a 0·1 mc injection of a ^{14}C-isotope in an ear vein or within 10 min. of oral administration in the feed. A. G. POLLARD.

Dicoumarol studies. IV. Antidotal and antagonistic properties of vitamins K_1 and K_3 in cattle. B. P. Goplen and J. M. Bell (*Can. J. Anim. Sci.*, 1967, **47**, 91–100).—Steers, fed a normal ration of lucerne hay and grain were treated with Dicoumarol [3,3′-methylene-bis-(4′-hydroxycoumarin)], (*D*), alone or with vitamin K_1 or vitamin K_3. Orally administered K_3 was an effective antagonist to *D*. In massive doses intravenous K_1 and oral K_3 showed high antidotal activities, K_3 having much the smaller efficiency. Best results in the treatment of 'sweet clover disease' were obtained with a massive dose (2000 mg) of K_1 given intravenously. By this means prothrombin times returned to normal in ~24 h, with min. 'rebound'. A massive oral dose of K_3 although less effective was still a useful antidote. A. G. POLLARD.

Nutrition of Zebu cattle in Northern Nigeria. II. Carotene supplements. T. B. Miller and L. E. Iduma (*Expl. Agric.*, 1967, **3**, 287–293).—Heifers receiving a high level of supplementary groundnut cake and sorghum meal showed increased wt. gains due to addition of carotene (as red palm oil) to the diet, whilst animals receiving low levels of supplements showed no response to carotene addition. Feeding cottonseed enhanced the rate of depletion of carotene (vitamin A) in the liver. Carotene supplementation of the cows' diet before and after calving failed to improve the vitamin A potency of milk. A. M. CORNFIELD.

Intake and digestibility of nitrogen-fertilised grass hays by wethers. C. D. T. Cameron (*Can. J. Anim. Sci.*, 1967, **47**, 123–125).—In digestion trials, the effects of applying different levels of N fertilisers to the grass on the intake and digestibility of the mature hay are examined. The rate of application of the fertiliser had little effect on the dry matter intake, but increased the digestibility of the protein and decreased that of the dry matter and the N-free extract. Differences between the effects of the fertiliser on the intake and digestibility by sheep and by steers were insignificant.
A. G. POLLARD.

Hatchability of chicken eggs with fractured shells after storage for up to forty-two days in nitrogen. F. G. Proudfoot (*Can. J. Anim. Sci.*, 1967, **47**, 115–122).—Effects of shell fractures (hair-line and basket shell) on the hatchability of eggs after storage in air or in N_2, are examined. Patching basket shells with adhesive tape, and storage in N_2 improved hatchability. Ill effects of basket cracks were greater when these occurred in the equatorial area of the shell than when at the large end of the egg. Patching hair-line cracks was not advantageous but storage in N_2 increased hatchability. Use of plastic tape gave best results for eggs stored up to 21 days but collodion was more effective with eggs stored for longer periods. A. G. POLLARD.

[A] Effect on hatchability of shipping eggs after storage for different periods of time in a nitrogen gaseous environment. [B] Advance note on the hatchability of chicken eggs stored small end up. F. G. Proudfoot (*Can. J. Anim. Sci.*, 1967, **47**, 137–138, 142–143).—

[A] Eggs were stored for 7, 14, 21 or 28 days, packed in cardboard boxes and flushed with N_2 in a cooler at 10° and ~75% R.H. They were turned daily. Subsequently some eggs were removed and despatched by motor transport (approx. 100 miles) and then by air from Halifax, N.S. to Ottawa with intermediate transshipment. Hatchability after storage for 7 to 28 days was compared with that for similar periods of storage followed by transportation. Eggs stored initially for ⩾14 days showed no ill effects on hatchability due to transportation.

[B] Eggs stored with small ends up as in [A] for 7–14 days showed no significant effects on hatchability due to position in storage. Evidence was obtained that eggs stored with small ends upward for ⩾7 days hatched better than did those stored with small ends downward. For eggs stored >14 days, the reverse was the case. A. G. POLLARD.

Rapeseed meal in rations for breeder turkeys. A. R. Robblee and D. R. Clandinin (*Can. J. Anim. Sci.*, 1967, **47**, 127–130).—Rations containing 10% of solvent-extracted rapeseed meal are compared with isonitrogenous, isocaloric feeds for breeder turkeys. The introduction of the rapeseed meal had no ill-effects on the mortality, rate of egg-production, fertility, hatchability or market quality of the turkeys at the end of the breeding season. In one of two trials the use of rapeseed meal increased the amount of feed required per dozen eggs produced. The NH_2-acid distribution of the rapeseed ration was similar to that of the control ration. A. G. POLLARD.

Utilisation of rapeseed meal as a source of protein for turkeys. J. D. Summers, W. F. Pepper, E. T. Moran, jun. and H. S. Bayley (*Can. J. Anim. Sci.*, 1967, **47**, 131–136).—Starter rations, containing 10, 20, or 30% rapeseed meal, (**I**), were fed to turkeys from 1 day of age. At 56 days a linear decrease in wt. gains and feed utilisation as compared with an isonitrogenous, isocaloric maize–soya-bean basal ration, was apparent. Steam pelletting the diets reduced the wt.-depressing effect of the **I** to below that of a similar diet fed as mash. At 113 days, the levels of **I** were reduced to maintain suitable levels of dietary protein, but the gain in wt, and feed utilisation still remained lower in birds fed **I** than in controls. Most of the adverse effects of **I** on growth were found in birds up to the age of 56 days. A. G. POLLARD.

Toxicity of timber milkvetch, *Astragalus miser* var. *oblongifolius*, to animals. M. C. Williams and W. Binns (*Weeds*, 1967, **15**, 359–362).—Timber milkvetch was most poisonous to chicks and cows during the vegetative and early bloom stages, but toxicity did not disappear until the leaves became bleached at senescence. Plants treated with 2,4,5-T (2,4,5-trichlorophenoxyacetic acid, 2–4 lb) or silvex [2-(2,4,5-trichlorophenoxy)-propionic acid, alkyl amine salt, 2–4 lb per acre] were less poisonous to cattle than were untreated plants. A. H. CORNFIELD.

Cattle grub control by dust bag application of coumaphos in summer. J. G. Matthysse, J. E. Lloyd, J. F. Butler and K. Tillapaugh (*J. econ. Ent.*, 1967, **61**, 311–312).—Self-treatment with 5% coumaphos dust at 0·05 lb/head per day for 44 days reduced grub infestation in 10 of 11 calves. Pour-on Ruelene treatment produced no extra protection. A 5% fenthion dust was ineffective. C. M. HARDWICK.

Some relationships of face fly feeding, ovarian development and incidence on dairy cattle. T. A. Miller and R. E. Treece (*J. econ. Ent.*, 1968, **61**, 250–257).—A relationship was found between the periods when face flies were found on the faces of cattle, their responses to certain foods and ovarian development. (32 references.) C. M. HARDWICK.

Preliminary evaluation of heliotrine as a sterility agent for face flies. H. M. Zapanto and C. W. Wingo (*J. econ. Ent.*, 1968, **61**, 330–331).—A solution of heliotrine (2 μg/ml) reduced oviposition and egg hatch by 28 and 6% respectively for two generations, without affecting longevity. At concn. >43·4 μg/g dry manure it was very toxic to larvae but not eggs. Formation of eggs in the ovaries was inhibited 60%. C. M. HARDWICK.

Inhibitive effect of *Bacillus thuringiensis* on the development of the face fly in cow manure. A. A. Hower, jun. and T.-H. Cheng (*J. econ. Ent.*, 1968, **61**, 26–31).—Cows fed bacillus spores as 1·25% of their daily diet, showed a reduction in the no. of *Musca autumnalis* developing from egg to adult in their manure of 99·6%. At 0·625%, development was reduced 84%, inhibition being between egg and pupae. When larvae were added to manure, adult emergence was reduced 97·1% and 42·3% resp. for the two dosages. The presence of spores was not a deterrent to fly oviposition. C. M. HARDWICK.

Some effects of orally administered Ruelene on host sheep and two ectoparasites: the yellow-fever mosquito and the Rocky Mountain wood tick. H. G. Smith, jun. and R. L. Goulding (*J. econ. Ent.*, 1968, **61**, 292–297).—Feeding of sheep with Ruelene depressed their cholinesterase (**I**) activity after 3 h. No toxic effects were apparent up to 48% depression. Mosquito mortality occurred when their **I** inhibition was >33%, with 100% mortality at 56%. There was no mortality of *Dermocentor andersoni* removed from sheep at 0–3 h but 80–100% after 9 h. (12 references.) C. M. HARDWICK.

Sheep ectoparasite control. II. Toxicity to sheep, and residues of diazinon and lindane. J. G. Matthysse, W. H. Gutenmann and R. Gigger (*J. econ. Ent.*, 1968, **61**, 207–209).—Dipping lambs in 0·06% diazinon gave residues in the fat after 1 but not after 15 days. Sprinkling caused lower residues. Of 12 sheep dipped, only one showed toxic effects. Sheep dipped twice in 0·06% lindane gave 44 ppm in fat after 2 weeks. (12 references.) C. M. HARDWICK.

Dust containing *Bacillus thuringiensis* for control of chicken body, shaft and wing lice. R. A. Hoffman and R. E. Gingrich (*J. econ. Ent.*, 1968, **61**, 85–88).—Dust prep. of *B. thuringiensis* (Bakthane) were effective against *Menopon gallinae* and related spp. on white Leghorn hens. Direct application to the birds was more effective than the addition to dust baths. C. M. HARDWICK.

2.—FOODS

Carbohydrate Materials

Cereals, flours, starches, baking

Association of enzyme proteins with starch granules in rice grains. Y. Tanaka, S. Minagawa and T. Akazawa (*Stärke*, 1967, **19**, 206–212).—Association of the enzyme amylose synthetase with rice starch granules has been studied to reveal the nature of the bonding. The protein was not liberated by treatment with 0·1 M Tris buffer, 4·0 M urea or 6·0 M urea. On treatment with 8·0 M urea or 5·0 M guanidine-HCl, a sharp decrease in enzyme activity occurred at the same time as the cryst. structure of the starch granule broke down, as indicated by *X*-ray diffraction and viscometry. A UDPG-pyrophosphorylase enzyme capable of incorporating glucose-^{14}C from glucose-1-phosphate into starch in the presence of UTP was readily dissociated with 0·1 M Tris buffer. Association of these enzymes with starch is considered to be significant in biosynthesis. (24 references.) (In English.) J. B. WOOF.

Early-generation screening for gluten strength in Durum wheat breeding. V. M. Bendelow (*Cereal Sci. Today*, 1967, **12**, 336–337).—A rapid procedure is described in which 30-g samples, previously tempered to yield 14% moisture, are first milled for 12 min. in an Ottawa micro flour mill (cf. Kemp *et al.*, *Cereal Chem.*, 1961, **38**, 50); the flour stock passing the 75 × 75 sieve (10 g) is then mixed with water (6 ml) and tested for strength in the electronic recording dough mixer (*cf.* Voisey *et al.*, *Cereal Chem.*, 1966, **43**, 408). The procedure permits the discarding of unsuitable strains at an early stage. P. S. ARUP.

Storage of flours in the biscuit and bread industries. K. Weber (*Industrie Aliment., Pinerolo*, 1967, **6**, 81–84, 86, 87).—Recent developments in storage silos for flours, etc., in large bakeries and biscuit factories are described; layout diagrams and typical plants are illustrated. C. A. FINCH.

Physicochemical studies on starches. XXXII. The incomplete β-amylolysis of amylose: its cause and implications. W. Banks and C. T. Greenwood (*Stärke*, 1967, **19**, 197–206).—The β-limits of amyloses from a number of botanical species are always much less than 100%; the barrier to further degradation is found in only a fraction of the mol. The actual limit depends upon the maturity of the starch. Physical properties of the amylose β-limit dextrins are found to be completely different from those of amylopectin β-limit dextrin. The possible effects of aggregation, and the presence of oxidised glucose units, of ester phosphate groups and of branch points have been investigated. Z-enzyme is shown to be merely a weak α-amylase and does not specifically remove the barrier to further amylolysis. Since pullulanase and β-amylase together cause complete degradation, the amylose must contain a limited number of α-1 : 6 linkages. (27 references.) (In English.) J. B. WOOF.

Protease enzymes; effect on bread flavour. A. A. El-Dash and J. A. Johnson (*Cereal Sci. Today*, 1967, **12**, 282–283, 286–288).—Max. improvements in the texture, grain, and aroma of bread were

obtained by additions of papain at 300 haemoglobin units (H.U.) per 700 g of flour or of Rhozyme J-25 at levels up to 100 H.U. The improvements were accompanied by increases in the carbonyl compounds in the crust, and in crust colour. Larger additions of papain were deleterious. P. S. ARUP.

Use of Cottage cheese whey solids in sponge bread. E. J. Guy, H. E. Vettel and M. J. Pallansch (*Baker's Dig.*, 1967, **41**, 44–50).—Baking and other tests have been carried out to determine the effect of replacing non-fat dried milk with dry Cottage cheese whey. Substitution of one-third of the usual non-fat dried milk in a 6% milk bread permitted greater tolerance to absorption variation, increased grain score, extended the mixing requirements of breads, improved the keeping quality and produced bread of good taste acceptability. It maintained equal and better vol. and bread scores for hard red winter wheat flour at intermediate and long mixing times but lowered the vol. of hard red spring wheat flours without decreasing the total score. S. A. BROOKS.

Sterilisation of sliced bread. C. Dietl (*Industrie Aliment., Pinerolo*, 1967, **6**, 93–96).—Possible infections of sliced bread are discussed, and sterilising methods described. Possible packaging materials are outlined briefly. The use of commercial sterilising ovens is shown. C. A. FINCH.

Influence of whey solids on cake quality. B. W. Best (*Baker's Dig.*, 1967, **41**, 38–76).—Cakes made with varying amounts of whey substituted for, and used in combination with milk solids, were prepared at five shortening and three sugar levels. A very good cake with greater vol. was obtained with whey solids and a decreased amount of shortening. Cakes made with whey were very tender, had a velvety texture and a resilience to pressure and were stable to handling; they gave low compressimeter readings both before and after freezing. S. A. BROOKS.

Metallo monoalkyl succinates as starch modifiers. Chas. Pfizer and Co. Inc. (B.P. 1,077,988, 6.9.65. U.S., 7.4.65).—The staling rate of baked leavened dough products, is retarded by including 0·1–3% by wt. of a Na, K, or Ca salt of a higher alkyl acid succinate (14–20C in the alkyl) in the dough. S. D. HUGGINS.

Puffed food product. Kellog Co. (Inventors: J. J. Thompson and C. A. Doan) (B.P. 1,068,410, 20.10.65).—Cereal dough containing 5–25 wt.-% of sugar is admixed in particle form with 1·5–5 wt.-% of solid hydrogenated vegetable oil and the particles are then explosively puffed, to give a food product of improved eating properties, storage stability, and ability to remain crisp when combined with milk. F. R. BASFORD.

Cereal food product. General Mills Inc. (B.P. 1,077,334, 21.1.66. U.S., 1.2.65.)—The title product is made by extruding a dough material (water and cereal flour, e.g. of corn, rye, oats, rice, wheat) to form a strand of pre-determined cross-sectional shape and pattern, and then stretching the extruded material along its longitudinal axis to reduce the cross-sectional area. Thus, a ground mixture of 87% yellow corn cones, 9·7% sugar and 3·3% NaCl is mixed with water to a moisture content of 22–35% by wt. The mixture is then cooked to a dough which is extruded into strands that are cohered and locked to form an extruded rope, which is finally stretched to reduce the diameter to 0·3125 in. These extruded lengths can be tempered, further cut or treated by cooking and puffing. S. D. HUGGINS.

Anhydrides of acidic lipids. Procter and Gamble Co. (B.P. 1,067,686, 4.11.64. U.S., 4.11.63).—Useful as additives for bakery products, the title compounds include stearoyl propylene glycol succinate anhydride (**I**), propylene glycol monoester glutarate anhydride in which the fatty acid radicals have 12–22C, fatty alcohol succinate anhydrides in which the alkyl radicals have 11–21C and fatty alcohol glutarate anhydrides in which the alkyl radicals have 11–21C; the dicarboxylic acid is esterified with the fatty alcohol in a mutual solvent at 75–175°, in the optional presence of a catalyst such as H_2SO_4, HCl, $ZnCl_2$. Thus, stearoyl propylene glycol hydrogen succinate and Ac_2O are refluxed for 1 h and then heated at 120–130° for 2 h with reduction of pressure to a final range of 2–5 mm. The residue is cooled to give a 96% yield of **I**, min. m.p. 41°, complete m.p. 77·7°, saponification value 385. S. D. HUGGINS.

Manufacture of bread. Ormeau Bakery Ltd. (Inventor: R. T. McHenry) (B.P. 1,067,161, 5.3.63).—Apparatus is diagrammed and claimed; it is designed to obviate or mitigate the manual or doubling operation. F. R. BASFORD.

Sugars and confectionery

Experience with the plate evaporator in the corn starch and glucose industries. R. M. Gray (*Stärke*, 1967, **19**, 219–224).—The development and present design of plate evaporators are reviewed. Some applications are described in detail. (In English.) J. B. WOOF.

Volatiles from the thermal degradation of glucose. R. H. Walter (*Diss. Abstr.* B, 1967, **28**, 228).—Chemical dehydration, pH changes and evolution of carbonyls and CO_2 were studied during low-temp. pyrolysis of glucose (**I**) in atm. of air and N_2. The volatile fraction obtained at 250° for 30 min. was analysed by use of a coupled capillary gas chromatographic–mass spectrometer unit; products from low-temp. pyrolysis of **I** appear to be independent of the heating atm. At least 100 volatile compounds were recovered by the method of distillation and concn. employed. The existence of phenol as a volatile compound from heated **I** is of special interest, due to its known toxic properties, whilst the presence of the γ-lactone of 4-hydroxy-2-pentenoic acid is noteworthy, because of the implication of such lactones in antibiosis. An enolic isomer of another component, 3-methylcyclopentane-1,2-dione, has been reported to impart a maple flavour to food products. F. C. SUTTON.

A process for the recovery of diffusion juice from sugar-beet by intermittent and continuous operation, and the difference in the quality of juice. B. Rogina, I. Brozović, I. Milostić and V. Jović (*Kemija Ind.*, 1967, **16**, 281–285, 295).—The purity of sugar-beet juice obtained from diffusion battery, continuous process is compared with that from diffusion tower, intermittent process. Battery juice was of higher purity. T. M. BARZYKOWSKI.

Separating sugars. Rubicon G.m.b.H. (Inventor: J. B. Stark) (B.P. 1,073,284, 11.2.65).—Sucrose is separated from a sugar mixture, especially cane and beet molasses, by ion exchange resins and eluted with water before equilibrium is reached between the liquids inside and outside the resin, so as to give effluents, with an increased sucrose concn., which are worked up. Thus, cane molasses are diluted with water and fed into a 100 cm column of a resin consisting of a sulphonated copolymer of styrene with approx. 4% di-vinyl benzene, (K^+ form) of 50–100 mesh. The column is then immediately washed with water at 90° and each fraction analysed for sucrose (greatest quantity and purity in fractions 13 and 14), while the reducing sugars present in the original molasses are obtained in highest concn from fractions 15 and 16. S. D. HUGGINS.

[Manufacture of] chewing gum. Frank H. Fleer Corp. (B.P. 1,081,015, 25.11.64. U.S., 2.12.63).—Dry sugar particles are added to a dry, molten chewing gum base, at an initial temp. of 77–121°, and mixed until a dry, friable, pulverulent mixture is formed. The gum base forms 5–40 wt.-% of the final product, the balance being mainly sugar, with moisture <1%. J. M. JACOBS.

Fermentation and Alcoholic Beverages

Fermentation of glucose by *Bacillus alvei* and *Bacillus circulans*. L. de Mey, R. Morel and A. Wilssens (*Revue Ferment. Ind. aliment.*, 1967, **22**, 169–176).—Yields of the following products of anaerobic fermentation at 35° and at controlled and uncontrolled pH, are tabulated: CO_2, formic, acetic, and lactic acids, EtOH, glycerol, butane-2,3-diol and diacetyl; the % of glucose fermented during 5–8 days (in the Neish medium with added yeast extract) are included. The results indicated primary fermentation by the Embden-Meyerhof pathway and secondary fermentation by the hexose-monophosphate pathway. In experiments with Na pyruvate as substrate, 64–92% of the pyruvate was degraded during 7 days. (16 references.) P. S. ARUP.

ZDBT colorimetric method for the determination of copper in alcoholic products. D. H. Strunk and A. A. Andreasen (*J. Ass. off. analyt. Chem.*, 1967, **50**, 334–338).—Collaboration work shows that the Zn dibenzyldithiocarbamate colorimetric method gives results of good precision. Colour measurement at 438 nm is recommended. A. A. ELDRIDGE.

Comparison of gas chromatographic methods for the investigation of aromatics in beverages, also description of solvent extraction [process] for aromatics in beverages. K. Wucherpfennig and G. Bretthauer (*Z. analyt. Chem.*, 1967, **228**, 342–360).—An apparatus is described which extracts the aromatics from beverage into an

ether–pentane (2 : 1) mixture. The extract is then subjected to gas chromatography. The chromatograms differ considerably from those obtained by direct chromatography of the beverage, or from the aroma-vapour. It is possible to concentrate the aromatics in the extract by evaporating the solvent.
A. TOWNSHEND.

Uptake, storage and utilisation of phosphate by [brewers] yeast. II. Limiting factors of yeast growth. E. Markham and W. J. Byrne (*J. Inst. Brew.*, 1967, **73**, 271–273).—Yeast (**I**) can continue to grow after it has used all the phosphate (**II**) in the medium because it utilises endogenous P reserves. No diauxic phenomenon was observed during the period of growth at the time the exogenous **II** concn. became zero. No evidence was found for the presence of any inhibitory substances secreted by **I** during its growth. **I** growth was clearly nutrilite limited. In a medium containing 65 μg P per ml and 5% glucose (**III**), the yield was limited by the **III** concn. and not by the **II** concn. **III** was found to be the sole limiting nutrilite. I. DICKINSON.

Initiation of specific metabolic pathways in yeast cells by fatty acids. W. Nordheim (*Mschr. Brau.*, 1968, **21**, 28–35).—Baker's yeast has been investigated since it adapts to anaerobic conditions, whereas other yeasts are predominantly respiratory or strongly fermenting. It has been shown that unsaturated fatty acids (**I**) like petroselinic, oleic, erucic, linoleic, linolenic, arachidonic and clupanodonic can promote metabolism whereas the saturated acids palmitic and stearic are without effect; **I** also delay the decline in metabolic activity. The magnitude of these effects is not directly related to the number of double bonds. The effect of **I** is to activate latent ATP-ase so that increased concn. of inorganic phosphate and ADP are available. In normal cells, external Mg^{2+} which is known to activate ATP-ase has little effect but with specially grown cells low in Mg it can increase fermentation rate even in absence of **I**. The increase in fermentation induced by **I** cannot be reversed by bovine serum albumin; indeed this protein has a beneficial effect also. (54 references.) J. B. WOOF.

Phytochemical studies on New Zealand hops. I. Comparison of essential oils by gas–liquid chromatography. J. R. L. Walker (*N. Z. Jl Sci.*, 1967, **10**, 476–480).—The steam-volatile oil (0·3–1·2 ml per 100 g) from each sample was chromatographed on a 15-ft column of 10% Carbowax 20-M on Chromosorb P, temp.-programmed from 50° to 150°, with N_2 as carrier-gas and dual compensating flame-ionisation detectors. Chromatograms for eight New Zealand and four European varieties of hop show that each has a distinctive 'fingerprint' by which it can be identified. Oils from European hops differ markedly in composition, having a high concn. of methyl-4-decenoate; New Zealand hop oils, are high in 2-methylbutyl isobutyrate and (some varieties) in β-selinene. Oil yields and concn. of some components, e.g. myrcene, vary with crop ripeness, but the 'fingerprints' are unaffected. (15 references.) W. J. BAKER.

Isomerisation of humulone. III. Degradation of isohumulone. B. J. Clarke and R. P. Hildebrand (*J. Inst. Brew.*, 1967, **73**, 282–293).—Humulone (I), iso-**I**-A (**II**) and humulinic acid (**III**) are examined by GLC, mass spectrometry and ozonolysis. **I** or **II** with aq. alkali form **III**, **II** being an intermediate. The final conversion is due to the rupture of the isohexenoyl side chain at C_4. A by-product is a six-carbon fragment recovered as 4-methyl-pent-3-enoic acid. It is shown that **II** can be fractionated into an oily dextrorotatory form A1 and a cryst. laevorotatory A2 which is identical to photo-isohumulone. The position of allo-isohumulone is discussed. (47 references.) I. DICKINSON.

The fate of amino-acids during mashing and hop boiling. M. Jones and J. S. Pierce (*J. Inst. Brew.*, 1967, **73**, 342–346).—In an infusion mash system the concn. of all α-amino-acids in sweet wort reaches a max. value when approx. $\frac{1}{3}$ of the total vol. extracted has been run from the mashing vessel. The relative concn. of each α-amino acid in wort vary throughout the mash extraction owing to differences in rate of extraction of the amino-acids and to the quantities produced by peptidase activity in the mash tun. The relative proportions of proline, alanine, glutamine and asparagine in the total α-amino-N content of wort decrease appreciably as the mashing process proceeds. Copper boiling results in the partial destruction of methionine, glutamic acid, lysine, arginine and valine present in sweet wort together with an increase in concn. of NH_4^+ in the wort. S. A. BROOKS.

Paper strip analysis of substances derived from hops. M. Verzele, H. Claus and J. Van Dyck (*J. Inst. Brew.*, 1967, **73**, 298–306).—A chromatographic technique using buffered paper strips is described. It is applicable to hop extracts, isomerised or not, and also to hops and beer. The percentage of α-acids and iso α-acids can be determined in one analysis; the determination of humulinic acids, β-acids and hulupones may also eventually be possible. After separating these substances from one another, they are determined spectrophotometrically. (13 references.)
I. DICKINSON.

Copper content of hop extracts. H. Weyh (*Brauwissenschaft*, 1968, **21**, 56–60).—The copper content of nine hop extracts was determined by the Trommsdorff method (*Mschr. Brau.*, 1962, **15**, 78). When the content of bitter substances was taken into consideration based on an average of 15% total resin content, and the results were compared with those published for hops from regions in which the extracted cones were grown, it was found that according to the desired concn. of bitter substances in the extraction, only a part (more or less significant) of the copper found in the hops is carried through into the extracts. (14 references.)
I. DICKINSON.

Barley variety, cultivation conditions, wort composition, fermentation pattern. Part II: The carbohydrate pattern of the wort as a genetic property of the barley variety. F. Weinfurter, G. Fischbeck, F. Wullinger, L. Reiner and A. Piendl. (*Brauwissenschaft*, 1968, **21**, 17–21).—The part which variety and environment play in the variability of barley was determined by variance analysis of the carbonhydrate levels of worts made from eight varieties which were grown in 17 different locations. It is concluded that the breeding of barley for brewing can contribute towards a high content of fermentable sugars in wort. (82 references).
I. DICKINSON.

Barley and malt analysis and brewhouse extract. G. Krauss and C. Kremkow (*Mschr. Brau.*, 1968, **21**, 5–9).—Using a computer it has been shown that the protein content of barley can be used to give a better estimate of brewhouse yield than the extract determinations of Graf and of Drinklage. The non-linear relationship was $Y = 1{\cdot}04\ P^2 + 21{\cdot}6\ P - 31{\cdot}6$ (where Y is the yield and P the protein content). From testing a series of brews it was found that for Miag-coarse grind (25 and 40%) the fine grind extract of malt gave a better indication of brewhouse behaviour than either the coarse grind or the fine–coarse difference. With the EBC mill a better prediction could be obtained by taking into account both fine and coarse grind extracts. The relationship found was $Y = 38{\cdot}7 + (F + G)/4$ where Y is the yield and F and G the fine and coarse grind extract values respectively. J. B. WOOF.

The Institute of Brewing recommended methods of analysis of barley, salt and adjuncts. (*J. Inst. Brew.*, 1967, **73**, 233–245).—Principal amendments relating to the following are given: (1) Thousand corn wt. (2) Extract prediction. (3) Moisture: more stringent limits are imposed in the $CuSO_4$ test. (4) Nitrogen content: distillation with boric acid and subsequent titration is the preferred method. (5) Colour: the colour determination adopted by the Institute of Brewing (*ibid.*, 1965, **71**, 476) is now incorporated. (6) Diastatic power: the use of Merck's sol. starch is now specified to improve uniformity. (7) A scheme for the presentation of findings is recommended. I. DICKINSON.

Melanoidins, their properties and rôle in malting and brewing. L. Petit (*Brasserie*, 1968, **23**, 9–12, 14–15, 17–20).—A review of Hodge's scheme for the mechanism of formation of pre-melanoidins and melanoidins and the possible effects of the process on malting and brewing. Attention is called to the 43 references in Dworschak's paper (cf. *Brewers' Dig.*, 1963, 63) on the same subject. P. S. ARUP.

Some quantitative aspects of the fermentation of maltotriose by yeasts. J. O. Harris and W. Watson (*J. Inst. Brew.*, 1967, **73**, 274–281).—Comparative fermentation velocities with glucose and maltotriose (**I**) of a series of different strains of top ale yeast show variation in the ratio of this function; flocculent yeast shows a relatively lower **I** fermentation rate, but this is not absolute. **I** fermentation rate is influenced by the N content of the cell. Media and conditions have been elaborated to yield a reproducible determination of the **I** fermentation efficiency and this characteristic strain parameter has been shown to be of value in strain evaluation. (14 references.) I. DICKINSON.

Molecular weight and soluble collagen content of finings in relation to fining potential. A. A. Leach and J. Barrett (*J. Inst. Brew.*, 1967, **73**, 246–254).—The fining efficiency of different samples of isinglass (**I**) finings is related to the mol. wt. of the finings molecules in true solution; those of highest mol. wt. are the most efficient. The mol. wt. is studied by intrinsic viscosity (η). Contributions from sol. aggregates are avoided by carrying out measurements in citrate buffer. Sol. aggregates occur in finings but do not con-

tribute to fining. The aim in the prep. of finings should be to produce the highest proportion of sol. collagen that is possible from a given amount of **I** whilst retaining a high μ. Both properties must be considered together; when η and the sol. collagen content are low, denaturation of the finings is indicated; if the former is low and the latter high, a poor quality **I** is denoted. (12 references.)
I. DICKINSON.

Effect of some foam stabilisers, detergents and oils on beer foam. G. Krauss, Ch. Zürcher and G. Jannaschk (*Mschr. Brau.*, 1968, **21**, 1–4).—The effects of seven foam stabilising prep., a quaternary ammonium compound (**I**), six detergents and six machine oils have been studied. After addition of 2 to 108 mg/l of the compound under test to the beer, head retention was measured by the Kolbach & Schilfarth modification of the Ross & Clark method, the surface tension by the method of Traube and also the viscosity η. One prep. had no effect but the others were satisfactory. There was an increase in η in one case but the surface tension was not changed appreciably. Detergents affected head retention only slightly but reduced cling to the glass; **I** had a disastrous effect on both. Mineral oils also had a detrimental effect. (29 references.)
J. B. WOOF.

Influence of malt modification on beer properties especially head retention and natural protein stability. [A] and [B]. E. Schild and K. Lempart (*Brauwissenschaft*, 1968, **21**, 1–11, 63–69).—[A] Trial brews carried out in a small-scale brewery are described. [B] Small scale trials are carried out to determine whether use of a moderately modified malt is more suitable for beer than a mixture of a very well and a very poorly modified malt. No definite results are found regarding pH and buffering. Mineral matter in beer increased with decreasing levels of modification, but this did not affect the quality of the beer. The protein content fell by 10 to 20% after mashing of poorly or very poorly modified malts. The viscosity η of beer rises when a moderately modified malt is used instead of a well modified one. 30% chit malt improves η and foam to an unusual extent; 30% barley does not produce this effect. Poorly and slightly modified malts lower the colour of the beer whilst raw barley does not. Malts which are moderately or normally modified yield beers with good natural protein stability and much better head retention than do well modified malts. Mashing of raw barley had no favourable results, whereas the use of chit malt in the grist had many advantages. (91 references).
I. DICKINSON.

Phenolic constituents of beer and brewing materials. I. Phenolic and nitrogenous components removed from beer by polyamide resins. J. W. Gramshaw (*J. Inst. Brew.*, 1967, **73**, 258–270).—An examination of nylon 66 and Polyclar A.T. adsorbates from beer by fractionation and paper chromatographic examination of the fraction is described. Aggregates of protein with polymerised polyphenols show marked resemblances to hazes and probably represent precursors very close to beer turbidities. The aggregates, which contain methoxylated phenolic units, in addition to anthocyanogen residues, are present in dialysable and non-dialysable forms. (39 references).
I. DICKINSON.

Determination of volatile thiols in beer. D. M. Sainsbury and G. A. Maw (*J. Inst. Brew.*, 1967, **73**, 293–297).—These compounds are determined by Ikeya's method, (*Bull. Brew. Sci., Tokio*, 1964, **10**, 23); the thiols (**I**) are aspirated into Hg-acetate. Cd-acetate is unsuitable for the removal of H_2S since it combines with **I**. Using Zn-acetate in a separate absorption trap, H_2S and these volatile **I** compounds can be determined on the same sample.
I. DICKINSON.

Quantitative gas chromatographic determination of higher aliphatic and aromatic alcohols in beer. V. Arkima (*Mschr. Brau.*, 1968, **21**, 25–27).—Total fusel oil content of a number of Finnish and foreign beers (both top and bottom fermentation) has been determined spectrophotometrically using *p*-dimethylaminobenzaldehyde in concn. H_2SO_4. In addition, individual concn. of n-propanol, iso-butanol, amyl alcohol, β-phenyl ethanol and tyrosol were measured by gas chromatography. The sum of individual components agreed with the spectrophotometric value to within 2 mg/l. (12 references.)
J. B. WOOF.

Rapid method for the determination of oxygen in bottled beers. R. Hiefner and D. Burwig (*Brauwissenschaft*, 1968, **21**, 11–13).—The indigo-disulfonate method by Kipphan (*ibid.*, 1964, **17**, 336–346) is modified. 5 ml ampoules which contain 10 times the amount of indicator and dextrose used in the Kipphan syringe method, together with 2·5 ml glycerin, water and 2 ml of KOH 50% are placed into clear glass sample bottles which are put at random on the filler conveyor belt with the other beer bottles. The ampoule is broken by means of a small glass ball inside it, the solution diffuses uniformly throughout the beer and reacts with the dissolved O_2. 'Standard colour' bottles are used for comparison. The method may also be useful for application to food colouring mixtures.
I. DICKINSON.

Sensory analysis of beer [by assessing smell, taste and temperature]. E. Krüger (*Mschr. Brau.*, 1968, **21**, 10–12).—A brief review of some of the factors and chemical constituents affecting the flavour and aroma of beers. (27 references.)
J. B. WOOF.

Some aroma compounds produced by vinous fermentation. A. D. Webb (*Biotechnol. Bioengng*, 1967, **9**, 305–319).—The higher alcohols and esters reported as aroma constituents of wines, with special reference to the Nordström mechanism of ester formation during fermentation are reviewed. White Riesling grapes contain small amounts of esters but after fermentation the wine contains relatively large amounts. Data from California sherry suggest enzymatic, rather than chemical esterification, after fermentation. The Nordström mechanism is extended to explain the formation of some secondary amides. (41 references.)
J. B. WOOF.

Fermentation of wines of Marcillac. J. F. Jouret, C. Roson, P. Galzy and C. Plan (*Revue Ferment. Ind. aliment.*, 1967, **22**, 183–185).—The low vat temp. usual in this district do not favour the malo-lactic fermentation. An experiment is described in which this type of fermentation was achieved with a vat temp. of 18°–20° and the use of a lactobacillus culture the prep. of which is described.
P. S. ARUP.

A kinetic study of the alcoholic fermentation of grape juice. I. Holzberg, R. K. Finn and K. H. Steinkraus (*Biotechnol. Bioengng*, 1967, **9**, 413–427).—Batch fermentations of Delaware grape juice were carried out in a commercial fermentor at pH 3·6 and 4·05 and the kinetics of the process were calculated in both the exponential growth (*EGP*) phase and the stationary phase (*SP*). In *EGP* the equation $dP/dt + BP = A\ (\ln N/\mu) - C$ related product concn. P to the cell concn. N; μ = specific growth rate. Under the conditions employed, $B = 0{\cdot}0326$, $A = 0{\cdot}0135$ and $C = 1{\cdot}87$. In the *SP*, $dP/dt = B(P_m - P)$ (P_m = stoicheiometric const.) was used and performance in a continuous fermentor predicted. Alcohol was added to the grape juice to study the effect on yeast growth and was found to be without effect below 2·6 g/100 ml and to be completely inhibitory above 6·85 g/100 ml. In batch culture nutritional deficiency in addition to alcohol probably inhibits growth. (10 references.)
J. B. WOOF.

Determination of fixed acids in wines by gas–liquid chromatographic separation of trimethylsilylated derivatives. R. L. Brunelle, R. L. Schoeneman and G. E. Martin (*J. Ass. off. analyt. Chem.*, 1967, **50**, 329–334).—The trimethylsilyl deriv. of the acids (first pptd. as Pb salts) were separated in a glass tube 6 ft. × ¼ in. containing 3·8% SE-30 on Diatoport S, using He as carrier. Succinic, malic, tartaric and citric acids can thus be determined simultaneously. For tartaric acid, recoveries were 100, 110%, and by the A.O.A.C. method 104 to 114%.
A. A. ELDRIDGE.

Fruits, Vegetables, etc.

Blueberry growing. G. M. Darrow and J. N. Moore (*Fmrs' Bull., U.S. Dep. Agric.*, 1966, No. 1951, 33 pp).—Important species, highbush varieties and their culture, propagation, pruning, harvesting and marketing are discussed together with some notes on diseases and pests.
E. G. BRICKELL.

Growing raspberries. Anon. (*Fmrs' Bull., U.S. Dep. Agric.*, 1967, No. 2165, 14 pp).—Types, sites, planting, training and pruning, thinning, fertilising, cultivating, irrigating and harvesting are discussed with notes on diseases and insects and descriptions of the major varieties.
E. G. BRICKELL.

Cytotoxic effects of extracts from gamma-irradiated pineapples. Y. Mäkinen, M. D. Upadhya and J. L. Brewbaker (*Nature, Lond.*, 1967, **214**, 413).—The significance of cytotoxins induced by irradiation and of the ability of intact fruits to catabolise them is discussed. Cytotoxicity was evaluated from frequencies of mitotic and chromosome aberrations in onion-root cells treated with irradiated pineapple-juice. There was a high degree of detoxification of cytotoxins during 8 days' storage of pineapples irradiated with 30 and 50 kr, but negligible recovery of those irradiated with 100 and 500 kr, probably because of damage to the critical enzymes by the high irradiation doses. It is not thought that these cytotoxic and antimitotic properties correlate with any clinical toxicity of irradiated fruits eaten by humans or animals.
W. J. BAKER.

Coconut and Salmonella infection. C. P. Schaffner, K. Mosbach, V. C. Bebit and C. H. Watson (*Appl. Microbiol.*, 1967, **15**, 471–475).—Raw unprocessed coconut will support the growth of this organism which is particularly resistant to dessication. Original contamination is not due to carriers or polluted water but to contact with bacteria-containing soils, followed by dispersion via infected coconut milk and shells. Pasteurisation of raw coconut meat in a water bath at 80° for 8–10 min. was effective in killing such organisms without injuring the product; this step is now widely used in the coconut industry. (19 references.) C.V.

Acidity in certain canned spinaches. M. Decleire (*Revue Ferment. Ind. aliment.*, 1967, **22**, 177–182, 214–222).—The defect is due to an excessive development of acetic acid (probably derived from the pectins), and occurs chiefly in spinach that has been bleached at 65°–70°. Minor increases occur in the non-volatile acids, but these become greater during prolonged storage. Directions given for the prevention of the defect are efficient in the following (descending) order: dilution of the juice after blanching; squeezing and draining of the spinach after blanching, a supplementary blanching for 1 min. at 100°. The use of all the three methods is recommended. (10 references.) P. S. ARUP.

Treatment of hazel nuts, peanuts and soya beans. K.-H. Koch (B.P. 1,070,083, 23.1.65).—Neutralisation or modification of the taste and odour of the nuts and beans is achieved by soaking them (preferably after pre-heating to 30–110°) in an aq. solution of an acid mixture containing 0·001–1% by wt. (based on dry wt. of the nuts or beans) of at least two of the following acids: malic, lactic, tartaric and citric. The treated products (whole or ground) have a milder taste and smell. S. D. HUGGINS.

Synthetic potato flavoured crisps. Cely Enterprises Corp. (Inventor: F. Koon Sai Yee) (B.P. 1,084,979, 24.12.64).—The crisps are obtained by frying slices of a solidified mixture of tapioca flour, NaCl, water and potato granules, flour or ground flakes, in vegetable oil at 199°. The ratio of tapioca to potato is preferably 8 : 1–3 : 1, and the salt content is preferably 3%. S. D. HUGGINS.

Non-alcoholic beverages

Preserved soups. Unilever Ltd. (Inventors: H. Schmidt, C.-P. Ganssauge and W. J. Akkersdijk) (B.P. 1,066,176, 20.6.64 and 13.1.65).—In the packaging of soups, the components are assembled separately in sub-packages and the sub-packages are packaged together, the contents of one of sub-packages being preserved in the wet or moist state and the osmotic pressure increased to prevent the growth of micro-organisms. Thus, a vegetable soup with meat balls consists of 3 sub-packages: A, B and C. A contains pre-diced potato starch, NaCl, Na glutamate, hydrolysate, hydrogenated palm oil, peanut oil, onion powder, tomato flakes, white leek, green leek, parsley and noodles and the mixture is filled into an Al-coated paper bag, provided with a low pressure polyethylene foil, and then sealed but not sterilised. B contains carrots, peas, asparagus, green beans and vegetable cooking water and these vegetables are filled, in the air-dried state into an Al tube that is then sealed and sterilised. C contains meat balls, broth, fat, hydrolysate liquid, mono-glyceride and thickening agent; the meat balls are added to the other ingredients, which are emulsified and then the whole is filled mechanically into an Al tube for sealing and sterilising. S. D. HUGGINS.

[Production of] a soup-forming pasta. Compagnia Italiana Galles S.p.A. (Inventor: F. Scaccabarozzi) (B.P. 1,069,635, 30.6.64).—A pasta from which a soup is obtained with boiling water is prepared by mixing meat and/or vegetable extract, mono-Na glutamate (I), and one or more flavouring agents, adding at least part of the mixture to a desired flour (during or before kneading); then shaping. A typical composition (claimed) contains; I (11·4–30), NaCl (40–65), pulverised onion (5–11), celery seeds or white root (0·5), lactose (2–4·5), spices (1) and dried tomato powder (2–4%). F. R. BASFORD.

Tea, coffee, cocoa

Compatability of roast coffee with specific regard to coffee drinks. J. Wurziger (*Dt. LebensmittRdsch.*, 1968, **64**, 38–43).—The % extract and the alcohol sol. fractions of coffee are improved by fine grinding but the extract: ash ratio is greatest with medium grinding except where pressure filtration is used; the latter is the most effective extraction method. The extracts, pH values and titratable acidities of coffee blends, caffein-free and acid-free and purified coffees are given. The extracts, acidities and light petroleum extractables in relation to roasting conditions have also been studied. These conditions affect different varieties of coffee bean in different ways and to different extents though the chlorogenic acid content is relatively unaffected. Finally light petroleum sol. fractions from beans and extracts prepared in different ways are compared. J. B. WOOF.

Synthesis of ethyl-trimethyl-pyrazine, a new constituent of cocoa aroma. J. P. Marion (*Chimia*, 1967, **21**, 510–511).—The prep. of ethyl-trimethyl-pyrazine (I) as reference compound is outlined. Synthetic ways mentioned comprise (*i*) direct condensation of an α-diamine, made by reduction of the dioxime, with an α-diketone, (*ii*) ring-halogenation of trimethyl-pyrazine, (*iii*) side chain halogenation of tetramethyl-pyrazine and (*iv*) side chain metalation of tetramethyl-pyrazine with sodamide followed by reaction with MeBr, apparently the best method. I has a b.p. 85–90°/10 Torr; $n_D^{25°} = 1·4893$. (In English.) M. SULZBACHER.

Fermentation of tea leaf. Marshall's Tea Machinery Co. Ltd. (Inventor: F. A. Hopper) (B.P. 1,085,525, 20.1.64).—The fermenting apparatus consists of a means for introducing the leaves into a receiving zone, an endless conveyor, with a perforated conveying surface for passing the leaves to a leaf-discharging zone and a means of passing an O_2-containing gas through the conveying surface, to carry out the 'fermenting' (oxidation) process. S. D. HUGGINS.

Milk, Dairy Products, Eggs

Characterisation of the natural inhibitors in skimmilk affecting lactic acid bacteria. H. E. Randolph and I. A. Gould (*J. Dairy Sci.*, 1968, **51**, 8–15).—Three strains of *Streptococcus cremoris* (RI, HP, and KH), susceptible to the inhibitory action of normal milk, and one resistant strain of *S. lactis* (C2) were utilised in an investigation by immunological and fractionation procedures of natural inhibitors of lactic acid bacteria in skimmilk. Subcutaneous immunisation of rabbits and a cow with *S. cremoris* RI organisms resulted in marked specific increases in the inhibition and agglutination titres in the blood sera of both species and in the cow's skimmilk. Similar treatments with *S. lactis* C2 caused less specific and less pronounced increases in the blood sera titres of both species and had no effect on the titres of the cow's skimmilk. The inhibition and agglutination activity of skimmilk and whey for *S. cremoris* R1 was specifically removed by adsorption with antigenic prep. of the homologous culture. Fractionation of whey proteins from a cow immunised with *S. cremoris* R1 showed that the inhibitory factor was associated with the immune globulin fraction. The evidence appears to support the conclusion that the inhibitory action of skimmilk for lactic acid-producing streptococci is due, to a significant extent, to the presence of specific antibodies. (23 references.) M. O'LEARY.

Distribution of bacteria in milk drawn directly from the cow's udder. B. Bacic, H. Jackson and L. F. L. Clegg (*J. Dairy Sci.*, 1968, **51**, 47–49).—The arithmetic mean colony count of aseptically drawn milk from 97 cows was 3,403 bacteria/ml. The colony count was highest in foremilk but this had little influence on the count of the total milk because of the small vol. of foremilk. Cows in the first or second lactation had appreciably lower milk colony counts than cows in the third or subsequent lactation. M. O'LEARY.

Variation in cell content of milk associated with time of sample collection. I. Diurnal variation. J. W. Smith and W. O. Schultze (*J. Dairy Sci.*, 1967, **50**, 1083–1087).—Body cell content of quarter milk samples was shown to be lowest immediately prior to milking and highest during stripping and the succeeding 4 h and then to decline progressively until the end of the intermilking period. M. O'LEARY.

Relationship of phospholipids to enzymatic hydrolysis of milk fat. C. V. Patel (*Diss. Abstr.* B, 1967, **28**, 227).—Research was conducted to determine: (*a*) the inter-relationship of agitation, release of phospholipids (I) from the fat globule membrane, and hydrolysis of milk fat by the enzyme lipase in milk produced during various stages of lactation, and (*b*) the distribution of types of I, and the component fatty acids in the I displaced by agitation. TLC showed a uniform distribution of each class of I in cream and skimmilk of non-agitated and agitated milk. Among the three major fatty acids, C_{16}, C_{18}, and $C_{18:1}$ in I on the average constituted 25·85%, 14·16% and 32·60% respectively. F. C. SUTTON.

Rubidium and lead content of market milk. G. K. Murthy, U. Rhea and J. T. Peeler (*J. Dairy Sci.*, 1967, **50**, 651–654).—The average Rb content of market milk samples from various U.S. cities varied between 0·57 and 3·39 ppm with significant differences between cities. In general Rb contents of milk from inland cities was small and those on the coast large. Average Pb content varied between 0·023 and 0·079 ppm. There was no significant difference in the Pb content of milk of the various centres. (12 references.) M. O'LEARY.

Acid production and curd toughness in milks of different α_{s1}-casein types. A. M. Sadler, C. A. Kiddy, R. E. McCann and W. A. Mattingly (*J. Dairy Sci.*, 1968, **51**, 28–30).—Skimmilks from nine Holstein cows of the phenotypes A (1), AB (3), B (3), and BC (2) were inoculated with starter and renneted; both the time required to reach pH 5·0 and 4·8 and the toughness of the curd at both pH values were determined. Milk A was significantly ($P<0·01$) slower than B and BC in reaching pH 4·8. All differences in curd toughness were significant except between AB and B at pH 5·0. The curd was soft when α_{s1}-casein A was involved while C was associated with toughness. The wt. of separator slime per 454 g of milk separated was also determined and all differences between types were found to be highly significant. M. O'LEARY.

Bacteriological examination of grade A dry milk powder. H. E. Hall, D. F. Brown, H. M. Robinson, C. B. Donnelly and A. L. Reyes (*J. Milk Fd Technol.*, 1967, **30**, 219–221).—Some 223 samples taken for total, and coliform, count and presence of salmonellae, were examined and direct microscopic examination was made on 211 samples. No salmonellae were detected and bacterial count ranged from 260–1,300,000 per g, only 15·2% exceeding the 30,000 per g limit recommended by the Public Health Service. Coliform counts ranged from zero to 120 per g but only 0·9% exceeded the 90 per g limit set. About 20% of the samples showed an excessively high direct microscopic count. C.V.

Surface features of foam-dried milk powder granules from krypton adsorption measurements. E. Berlin, P. G. Kliman and M. J. Pallansch (*J. Dairy Sci.*, 1967, **50**, 659–663).—Comparison of surface area values, calculated from krypton adsorption data, of various milk powders indicated that the nature and vol. of gas injected during the foaming process had a marked effect on powder structure. Surface area size was shown to be directly proportional to the vol. of N_2 injected into the liquid milk concentrate prior to spray drying. With CO_2 and N_2O, a nonlinear relationship between surface area and injected gas vol. was observed. (11 references.) M. O'LEARY.

Comparison of toluene distillation and Karl Fischer methods for determining moisture in dry whole milk. E. S. Della Monica and T. F. Holden (*J. Dairy Sci.*, 1968, **51**, 40–43).—A comparison of the toluene distillation and Karl Fischer methods for determining the moisture content of dried whole milk indicated that in the 0 to 7% moisture range the toluene method is more precise. The results also indicated the existence of operator differences with both methods. M. O'LEARY.

New infant milk powder. T. Nagasawa (*Milchwissenschaft*, 1967, **22**, 537–539).—The addition of active mucin is described, the powder containing fat 22, protein 13, carbohydrates 60·8, ash 2·2 and moisture 2%. The powder is electrodialysed to free it from salt and contains almost the same electrolyte concn. as human milk. The presence of mucin accelerates fat and protein absorption and adjusts the flora of the intestinal tract. C.V.

Factors affecting stability of rennin. R. Mickelsen and C. A. Ernstrom (*J. Dairy Sci.*, 1967, **50**, 645–650).—Clotting activity of rennin was shown to decrease with increase in pH above 6·0 and was also highly dependent on temp. Max. activity was maintained from pH 5·0–6·0. A region of instability was observed between pH 3·0–4·9 in buffers of 0·03 ionic strength. An instability max. at pH 3·8 resulted in a 35% loss of initial activity at 30° over 76 h of incubation. This loss increased to 70% with increase of ionic strength to 1·0. Rennin was more stable in Na-citrate, Na-lactate, and $(NH_4)_2SO_4$ than in NaCl or KCl. Above pH 6·0 activity loss was accompanied by protein pptn. indicating pH denaturation; below pH 4·5 activity loss was accompanied by an increase in the amount of Ruhemann's purple formed by the action of ninhydrin on the rennin mixture, indicating self-digestion. (18 references.) M. O'LEARY.

Properties of caseins modified by treatment with carboxypeptidase A. M. P. Thompson, E. B. Kalan and R. Greenberg (*J. Dairy Sci.*, 1967, **50**, 767–769).—Details are given of the effects on α_{s1}-casein A and β-casein of the removal of C-terminal amino-acids by treatment with carboxypeptidase A. M. O'LEARY.

Solubilisation of cottage cheese curd by alkaline wash waters and its causes. H. T. Roth, R. T. Marshall and F. F. Schmidt (*J. Dairy Sci.*, 1968, **51**, 36–39).—The results of experiments indicated that the pH of the wash water must normally be as high as 11·0 before solubilisation or gelatination of cottage cheese curd will occur but that extremely hard waters may cause the defect at pH values as low as 9·0. It is recommended that cottage cheese curd should not be allowed to remain in alkaline water for any appreciable period. M. O'LEARY.

Phenolic flavour in cheese. H. T. Badings, J. Stadhouders and H. van Duin (*J. Dairy Sci.*, 1968, **51**, 31–35).—A phenolic type flavour defect in Gouda cheese was found to be due to the formation of *p*-cresol in the curd by lactobacilli originating in inadequately filtered rennet. M. O'LEARY.

Detection of egg-white in commercial liquid yolk. K. H. Ney (*Fette Seifen AnstrMittel*, 1967, **69**, 794–795).—A paper electrophoretic method for the detection of 10 to 15% of egg-white present in commercial egg yolk prep. makes use of the intensity of the ovalbumin (**I**) band, which is obtained by application of 0·01 μl of a 40% sample solution of **I** in a veronal buffer (of pH 8·6 and with ionic strength $\mu = 0·075$), on to a paper strip which is subjected to 200 V at 17 mA and run for 17 h. The strip after drying (30 min. at 120°) is washed with methanol, dipped in a 0·1% methanolic bromophenol blue solution, and washed with 5% aq. AcOH. The method is recommended for assessment of commercial egg yolk prep. G. R. WHALLEY.

Investigations on egg yolk gelation. W. C. Reinke (*Diss. Abstr.*, B, 1967, **28**, 228).—Analysis of the isolated egg yolk proteins and lipoproteins by disc electrophoresis indicated that LDF, lipovitellin, γ-livetin, α, β-livetins and phosvitin contained at least the number of components observed by other techniques. The effect of freezing and thawing egg yolk on the isolated proteins and lipoproteins resulted in an increase or decrease in the number of observed discs or an alteration in the rate of migration of the components. The postulated mechanism of egg yolk gelation involves phosvitin and/or Ca as the component responsible for the association of the various proteins with or without the low-density fraction. (LDF). F. C. SUTTON.

Cooling butter corn. Holstein und Kappert Maschinenfabrik Phoenix G.m.b.H. (B.P. 1,069,305, 24.11.65. Ger., 23.1.65).—Butter corn is cooled in continuously operating churns, where the butter milk is removed in a separating cylinder and cooled in a refrigerator, followed by spraying into a rechurning portion of the separating cylinder and/or in the gravity shaft between the churning and separating cylinder directly over the butter corn. A portion only of the separated butter milk can be used for cooling, while the remainder is discharged. S. D. HUGGINS.

Edible Oils and Fats

Suitability of extraction solvents for production of edible oils. C. Franzke, G. Heder and B. Klingberg (*Nahrung*, 1967, **11**, 277–284).—In laboratory experiments increasing amounts of aromatic hydrocarbons (3 to 15%) in the solvent (petrol) extracted increasing amounts of colouring matters with the oils from flaked sunflower and rape seed. The extracted oils were more difficult to decolorise and more prone to autoxidation than were oils extracted with hexane. The colours of raw and refined oils were compared by recording their absorption spectra between 410 and 750 nm spectrophotometrically by the author's method (*LebensmittelInd.*, 1966, **13**, 374). P. S. ARUP.

Meat and Poultry

Changes in food labelling regulations [in the German Federal Republic]. W. Zipfel (*Fleischwirtschaft*, 1967, **47**, 941–945, 948).—The changes came into force 1.1.68. The type of meat used must be stated, specially in the case of invented names or as in 'game pie'. This does not apply in the case of Mortadella (except where mutton or horse meat are used). The regulations relating to wt., date of manufacture, period of edibility and dating and wording of this information are stressed. (27 references.) C.V.

The meat basis of meat salad. K. Wenker (*Dt. LebensmittRdsch.*, 1968, **64**, 43–46).—Analyses of a number of samples of meat salad prep. have been carried out in order to establish quality standards. The following determinations are recommended: collagen content

in relation to total meat protein, residual water content, starch and milk protein content. Collagen contents of 16·4 to 31·6% were found and water varied from 10–30%. Starch and milk protein contents above the limit of 2% relative to the meat/fat content were very common. J. B. WOOF.

Dry sausage casing. Tee-Pak Inc. (Inventors: A. F. Turbak and H. J. Rose) (B.P. 1,086,604, 21.3.66).—The improved sausage casings for processing dry sausages are composed of regenerated cellulose tubing that has been coated on the inner surface with 0·1–5% of a sol. protein of mol. wt. >10,000 and isoelectric point of pH 2–6 (e.g. gelatin) and with sufficient liquid smoke (aq. wood smoke extract) in the range of 0·05–5% (on wt. of casing) to insolubilise the protein coating after drying, so that the casing shrinks with, and adheres to, the dry sausage during the processing. The coating may also include a non-toxic, anti-blocking powder (starch). The new casing avoids the formation of gaps between meat and casing on drying, thus discouraging formation of mould. H. L. WHITEHEAD.

Fish

Binding of oil in canned salmon. E. Bilinski and P. Clement (*J. Fish. Res. Bd Can.*, 1967, **24**, 1203–1207).—The vol. of oil separated from the drained liquor of canned salmon has been measured for salmon canned after various periods of cold storage. For fish stored 1–3 days on ice it was 2·1–2·5 ml/100 g fish; it decreased to 1·0–1·3 ml/100 g after 10 days storage on ice or 3–10 days in refrigerated, salt-fortified sea water at 25°F. Oil which was obtained by centrifuging, contained 65–70% of the total lipid, was not related to time of storage and was the same vol. as that from cans having a high drained oil content without centrifugation. Oil binding in canned salmon appears to be physical, rather than chemical; it may be related to protein denaturation during cold storage. (13 references.) S. A. BROOKS.

Bacterial effect of acetylated monoglycerides as protective coating on frozen fish. H. P. Dussault (*J. Fish. Res. Bd Can.*, 1967, **24**, 1179–1182).—Frozen samples of cod in various stages of freshness were dipped in Myvacet Type 7-00 (commercial distilled acetylated monoglycerides) at 160° for 12 sec and refrozen. After 24 h in cold storage total bacterial counts of samples were obtained. The observed inhibition caused by the coating procedure ranged from 71 to 92% and was greatest when initial contamination was most restricted. Bacterial survival was thought to be due to lack of heat penetration. (10 references.) S. A. BROOKS.

Factors influencing the nutritional value of fish flour. V. Chlorocholine chloride, a toxic material in samples extracted with 1,2-dichloroethane. I. C. Munro and A. B. Morrison (*Can. J. Biochem.*, 1967, **45**, 1049–1053).—Extraction of lyophilised cod fillets with 1,2-dichloroethane resulted in the formation of (2-chloroethyl)-trimethylammonium chloride (**I**), a toxic choline deriv. The compound isolated chromatographically from the fish was identical with that prepared by reacting 1,2-dichloroethane with trimethylamine; the i.r. spectra of the reineckate salts of both compounds were identical. A method for determination of **I** in 1,2-dichloroethane-extracted fish is described. S. A. BROOKS.

Freeze-drying for determining total solids in shellfish. W. N. Shaw, H. S. Tubiash and A. M. Barker (*J. Fish. Res. Bd Can.*, 1967, **24**, 1413–1417).—A method for determining the total solids in shellfish by freeze-drying is described in detail and the results compared with the oven-dry method. Freeze-drying was quicker and easier and gave results consistently slightly higher than oven-drying. Other methods of measuring shellfish condition are evaluated. (12 references.) S. A. BROOKS.

Effect of food additives and irradiation on the survival of Salmonella in oysters. M. A. Skiflett, J. S. Lee and R. O. Sinnhuber (*Appl. Microbiol.*, 1967, **15**, 476–479).—*Salmonella typhimurium* and *S. enteritidis* were inoculated into blended oysters both raw and autoclaved; the oysters were also treated with 0·1% of Na-benzoate, (**I**) or 0·1% of K sorbate, (**II**) and irradiated (0·1 Mrad). In both irradiated and non-irradiated samples, great numbers of Salmonella were found after storage at 7° in the presence of **I** and **II**. C.V.

Spices, Flavours, etc.

Traditional fermented foods. C. W. Hesseltine and Hwa L. Wang (*Biotechnol. Bioengng*, 1967, **9**, 275–288).—A general discussion concerning the traditional oriental foods, shoyu, miso, natto, tempeh, sufu, hamanatto, ontjom, katsuobushi and various fish products. The organism used, the flavour produced and the active constituents are described in each case. Many of the products are used as flavouring materials. J. B. WOOF.

Morel mushroom mycelium as a food flavouring material. J. H. Litchfield (*Biotechnol. Bioengng*, 1967, **9**, 289–304).—The factors affecting the flavour of mushroom mycelium are considered. Values for overall analysis, amino-acid composition, non-protein N constituents and essential oils are compared for different species. The highly basic amino-acids and the purine bases and nucleotides appear to be of major importance. Flavour depends on the strain used; some strains lack flavour even when grown on rich media; others require only minimal conditions. In general, media with complex N sources are preferred. Development of optimum flavour in submerged culture is also assisted by aeration and agitation. (61 references.) J. B. WOOF.

Methodology of flavour development before and after introduction of chromatography. D. Jorysch and J. J. Broderick (*Cereal Sci. Today*, 1967, **12**, 292–294, 312–313).—A review. (12 references.) P. S. ARUP.

Flavours and ingredients of interest to the cereal chemist. Anon. (*Cereal Sci. Today*, 1967, **12**, 289–290, 313–314.)—Brief descriptions are given of 24 proprietary prep., with five references to commercial literature. P. S. ARUP.

Food of increased amino-acid content. Kyowa Hakko Kogyu Co., Ltd. (B.P. 1,068,478, 4.8.64. Japan, 30.7 and 22.8.63).—Food, especially rice (but also seasoning, dairy product, a confection, animal fodder, etc.) is fortified by addition of ornithine-aspartic acid salt or peptide (0·1–5 wt.-%). Flavour of the food is also enhanced. F. R. BASFORD.

Flavouring compositions. International Flavors and Fragrances, Inc. (B.P. 1,084,619, 30.6.66. U.S., 7.7.65).—Food, particularly meat, has its flavour enhanced by treating with the compound of empirical formula $C_{10}H_{14}O_3S$, formed by the reaction of 3-acetyl-3-mercapto-propanol-1 or thiamine in presence of an org. carboxylic acid, e.g. unsubstituted aliphatic acid, $-NH_2$ or $-OH$ substituted aliphatic acid or a protein hydrolysate, at 180–350°F. Suitable acids are: glutamic, acetic, lactic, tartaric. Preferably an auxiliary S-containing compound (e.g. a S-containing amino-acid, an alkyl-mercaptan or -sulphide, or an inorg. sulphide) is also present during the reaction. S. D. HUGGINS.

***l*-Glutamic and α-ketoglutaric acids.** Kyowa Hakko Kogyo Co., Ltd. (B.P. 1,085,923, 18.8.65. Jap., 18.8.64).—Used in the manufacture of flavour enhancers, the claimed acids are obtained by culturing *Micrococcus paraffinolyticus*, *Brevibacterium ketoglutamicum*, *Arthrobacter roseoparaffinus* or *A. hydrocarboglutamicus* in an aq. nutrient containing at least one normal paraffin as the major C source and recovering the title acids produced. S. D. HUGGINS.

Cyclamic acid. Abbott Laboratories (B.P. 1,068,819, 8.2.66. U.S., 27.4.65).—A commercial process for prep. of cyclamic acid (cyclohexylsulphamic acid; sweetening agent for foodstuffs and beverages) of high (99·6%) purity comprises heating a mixture of cyclohexylammonium-*N*-cyclohexylsulphamate(100), water (300–550), and NH_2SO_3H (≯35·3 pt.) at 45–65° during ≮15 min., cooling to <25°, collecting cryst. material, and washing the latter with water (0·2–3 pt. per pt. of solid). F. R. BASFORD.

[Preparation of the magnesium] salt of cyclohexylsulphamic acid and [sweetening] composition containing it. Aspro-Nicholas, Ltd. (Inventors: J. R. Henderson and E. Pride) (B.P. 1,086,500, 30.12.64).—The Mg salt of *N*-cyclohexylsulphamic acid [Mg cyclamate (**I**)] is prepared by adding a basic Mg compound (oxide, hydroxide or carbonate) to an aq. solution of cyclamic acid, maintained at 80–100°, until a pH of 6·5–7·5 (7) is attained, and then evaporating to dryness on a steam bath. A sweetening composition comprises **I** with one or more solid ingestible carriers or aq. solutions containing 20–35% (wt./vol.). **I** has the advantage of increased water-solubility, and has no appreciable additional 'off-taste'. J. M. JACOBS.

Colouring matters

The use of polyamide in analyses of water-soluble food dyes. IV. Thin layer chromatographic separation of water-soluble food dyes. J. Davídek and E. Davídková (*J. Chromat.*, 1967, **26**, 529–531).—For isolation and separation of small amounts of synthetic food dyes, polyamide powder (~10 g) was mixed with a weak acid

extract of food dyes, or an acidified liquid sample of food, and filtered. The powder with adsorbed dye was washed with 50% AcOH solution to remove natural dyes. The synthetic dyes were eluted with 5% methanolic NH_3 and the eluate evaporated to dryness. The residue was separated by TLC on polyamide thin layers with NH_3–CH_3OH–water (1 : 3 : 16) as developing solvent. R_F values for 10 dyes are tabulated. Good separation was obtained for synthetic dyes from fruit jams and jellies, food preserves, canned fruits, beverages and sweet candies.
G. W. FLINN.

Preservatives

Hot water treatment and treatment with thiabendazole [2-(4-thiazolyl)-benzimidazole] against lenticular [fungal] parasites of apples. P. Bondoux (*C. r. hebd. Séanc. Acad. Agric. Fr.*, 1967, **53**, 1314–1323).—Promising results were obtained by dipping the fruit, before cold storage, in 0·4% or 1·6% aq. thiabendazole hydrochloride or lactate, the use of which, however, is forbidden in France. Results obtained with the hot-water treatment were much less satisfactory; this process is not readily adaptable for practical use. P. S. ARUP.

The protective effect of tocopherols on provitamin A (β-carotene) and vitamin A in enriched margarine and butter. L. T. Kováts and E. Berndorfer-Kraszner (*Periodica polytech.*, 1966, **10**, 513–522).—The antioxidative and protective effect of tocopherols (**I**) in margarine enriched with provitamin A (β-carotene) and in butter containing natural vitamin A has been studied. As antioxidants α- and γ-**I** and an extract from maize germ oil, containing mainly γ-**I** were used and citric acid, phosphoric and selenious acids were examined as synergists. The artificial ageing tests used u.v. irradiation and the active O_2 method. A **I** concn. of 20 mg % was the quant. optimum for the prolongation of the induction period, irrespective of the sort of **I**. A qual. optimum was achieved with the maize germ oil extract. As a synergist with 5 mg % of **I** mixture, selenious acid was best. The organoleptic properties of the fats were not affected. (16 references.) (In English.)
M. SULZBACHER.

Determination of propionic acid in foods by gas chromatography. M. H. Grosjean and A. Fouassin (*Revue Ferment. Ind. aliment.*, 1967, **22**, 211–213).—Propionic acid (**I**) is separated from the sample by steam-distillation (Reichert-Polenske apparatus). An aliquot of the neutralised, conc. distillate, suitable for GLC, is evaporated to dryness *in vacuo*, and the **I** is esterified by heating with PrOH and H_2SO_4 simultaneously with 15 mg of crotonic acid. The **I** is thus determined by GLC with propyl crotonate as internal standard. Recoveries of **I** from cheese or bread were complete, with a max. error of ~5%. (18 references.)
P. S. ARUP.

Determination of sorbic acid with sodium chlorite. P. Spacu and H. Dumitrescu (*Talanta*, 1967, **14**, 981–983).—After a preliminary ether extraction or steam distillation, sorbic acid is determined in food by addition of $NaClO_2$ followed by HCl whereby Cl_2 is added to the double bonds. Excess $NaClO_2$ is titrated iodometrically after addition of KI and starch. The relative error is ±6% but the method is rapid. F. C. SAVILLE.

Pesticides in Foods

Sweep co-distillation clean-up of fortified edible oils for determination of organophosphate and chlorinated hydrocarbon pesticides. R. W. Storherr, E. J. Murray, J. Klein and L. A. Rosenberg (*J. Ass. off. analyt. Chem.*, 1967, **50**, 605–615).—The rapid procedure (Storherr and Watts, *ibid.*, 1965, **48**, 1154) gives satisfactory recoveries (75 to 98% for chlorinated, 74–100% for phosphate pesticides). Prior extraction of the oil is not required and the resulting samples can be analysed by GLC using a K thermionic detector. A. A. ELDRIDGE.

Food Processing, Refrigeration

Variation of biological-nutritional value of foods during cooking and similar treatments. W. Ciusa (*Industrie Aliment. Pinerolo*, 1967, **6**, 106–109).—A general, historical review. Some details of recent work on changes in vitamin content of vegetables and cereals during cooking are given. C. A. FINCH.

Packaging

Polyolefin films. V. O. Smith (*Baker's Dig.*, 1967, **41**, 64–69).—The specific properties of the most common polyolefin films are described and their suitability for packaging bakery products discussed. S. A. BROOKS.

Absorption of moisture by banana cartons during storage and maritime transport. R. Deullin (*Fruits*, 1967, **22**, 273–277).—Exposure during 10 days of the dry cartons to a tropical atm. at 26°–30° and R.H. 100% increases their moisture content by 25–30%, the initial rate of absorption being comparatively rapid. Moisture absorption increases the thermal conductivity of the carton material (an important factor with respect to refrigeration) but decreases its mechanical strength. Increases in moisture during pre-refrigeration at 12°–13°, or in a ship's hold, were comparatively small. P. S. ARUP.

Changes in mycoflora of groundnuts stored at two temperatures in air or in high concentrations of nitrogen or carbon dioxide. C. R. Jackson and A. F. Press, jun., (*Oléagineux*, 1967, **22**, 165–168).—Cultural assays of groundnut pod surface mycoflora and kernel fungi were made before and during storage in high concn. of N_2 and CO_2 at two temp. Fungus populations on surfaces of pods of unshelled groundnuts were not significantly affected by storage gases. Significant decrease in no. was noted at 27° compared with that at 4° after storage for 6 and 12 months. Fungi, including *Aspergillus flavus*, cultured from stored shelled groundnuts were not significantly affected by storage gases but increased noticeably in no. after 12 months storage at 4°. Although fungus production was not significantly affected, high levels of CO_2 or N_2 appeared to reduce the formation of aflatoxin slightly. (12 references.)
M. DUDLEY.

Survey of temperatures involved in bottling milk in paper containers. A. C. Smith, L. R. Dowd and R. M. Parry (*J. Milk Fd Technol.*, 1967, **30**, 186–188).—The temp. during bottling in half-pint knock-down and preformed paper containers at ten dairy plants ranged from 43–56°F (average 47°F). The effects of these temp. on shelf life is discussed. The detrimental effect of great temp. rise of milk during bottling upon shelf life is apparent and demonstrates the inability to maintain milk temp. <65°F during bottling. Suggestions to overcome this are offered (*a*) use of lower temp. or greater vol. ratio of coolant to product, (*b*) increased cooling surface, (*c*) improved method of cooling knock-down paper containers after formation, (*d*) min. heating in formation of containers, (*e*) faster paper fillers and movement of packaged product to milk cooler. C.V.

Corrosion of aluminium caps by fruit flavoured yoghurt. G. Stehle (*Milchwissenschaft*, 1967, **22**, 374–378).—A report. C.V.

Transfer of lubricants from polystyrene into coconut fat. W. Pfab and K. Bernhart (*Dt. LebensmittRdsch.*, 1968, **64**, 33–37).—Use of ether and n-pentane to extract lubricants and oligomers from polystyrenes in order to give an indication of the amounts of contaminants which might reach food in contact with the plastic gives high values which suggest a possible high level of contamination in edible fats. To test this, coconut fat has been stored at 40° for 2 weeks in contact with samples of the plastic and the fat subsequently analysed for contamination by currently used additives, particularly paraffin oils, butyl stearate, dibutyl phthalate, octadecyl alcohol, di-isononyl adipate. Only negligible concn. of these compounds were found. J. B. WOOF.

Miscellaneous

Nutrition, proteins, amino-acids, vitamins

Influence of the type of carbohydrate in diets containing different levels of casein, vitamins and minerals on growth and body composition of the rat. A. C. Santos (*Diss. Abstr.*, B, 1967, **28**, 8–9).—Male weanling rats were fed diets with either dextrin (**I**) or sucrose (**II**) as the dietary carbohydrate combined in various ways with methionine-supplemented casein, a vitamin mix and a mineral mix, the two latter mixes being to NRC requirements. In diets limiting in protein, vitamins or minerals, **I** is superior to **II** in promoting growth. Neither **I** nor **II** influenced the moisture, protein and fat content of the rat carcasses, but ash content was lower with **I**. F. C. SUTTON.

Studies of lipid metabolism at several levels of organisation, and the influence of sucrose on energy-linked mitochondrial volume changes. R. W. Hubbard (*Diss. Abstr.*, B, 1967, **28**, 75).—Differences in the nitrogen, carbohydrate and lipid metabolism of

certain inbred strains of mice suggest that metabolic patterns are genetically determined in part through endocrine regulation of metabolism. Work on an A strain and an I strain mouse which showed differences between the strains in lipid metabolism, revealed that at all activity levels the I strain consumed significantly more (25%) O_2 than the A. The I strain oxidised 40% more orally-administered glyceryl tripalmitin-1-^{14}C to $^{14}CO_2$ than the A. Experiments were also performed on the influence of sucrose on mitochondrial respiration. F. C. SUTTON.

Amino-acid composition of heat-processed soyamilk and its correlation with nutritive value. L. R. Hackler and B. R. Stillings (*Cereal Chem.*, 1967, **44**, 70–77).—The effect of heat processing time and temp. (15–240 min. at 93°; 0–120 min. at 121°) before vacuum concn. to 16% solids and drying, and of spray-dryer inlet temp. (143, 188, 229, 277 and 316°) on the amino-acid composition, protein efficiency ratio (*PER*) and calculated essential amino-acid index (*EAAI*) and requirement index (*RI*) are reported. At 93° no significant changes in amino-acid composition, *EAAI* or *RI* resulted, but *PER* rose rapidly during the first 60 min. of treatment. At 121° *PER* rose rapidly during the first 5 min. of treatment and then gradually fell in general parallelism with *EAAI*, *RI* and degradation of amino-acids, particularly cystine, tryptophan and available (but not total) lysine. Spray-dryer inlet temp. was found to be critical, and temp. of 277 and 316° caused considerable losses of lysine, histidine, arginine, threonine, serine, proline, phenylalanine, tyrosine and tryptophan with reduction in *PER*, *EAAI* and *RI*. (11 references.)
E. C. APLING.

Solubility and ultracentrifugal studies on soybean globulins. A. M. Nash and W. J. Wolf (*Cereal Chem.*, 1967, **44**, 183–192).—Solubilities of five laboratory-prepared and five commercial samples of soybean globulins and of seven commercial soybean proteinate samples were measured in $K_2HPO_4/KH_2PO_4/NaCl$ buffer (pH 7·6; ionic strength 0·5) with or without 0·01 M 2-mercaptoethanol (**I**), and the compositions of the sol. protein were determined by ultracentrifugation. Solubilities of all samples were increased by **I**, but wide variations were found. Some commercial prep. resembled the laboratory prep.; others had low protein solubility and appeared to have been extensively modified by the isolation process. (23 references.) E. C. APLING.

Microbiological protein production from hydrocarbons. A. Fiechter (*Chimia*, 1967, **21**, 501–508). A review of technical protein production from petroleum hydrocarbons with yeast and bacteria covers the advantages, i.e., high protein yields and m.p. reduction of kerosine, fuel and diesel oils. The biology of the micro-organisms, especially of food yeasts (*Candida intermedia* and *C. lipolytica*), their growth and O_2 consumption are surveyed; influence of substrate chain length, solubility and constitution on degradability is discussed. Yields of up to 100% can be obtained on n-alkanes. Degradation normally starts from one end of the carbon chain; biterminal oxidation to dicarboxylic acids which are then subjected to beta degradation is also possible. The content of protein and lipids in the micro-organism cell alters with substrate and O_2 supply. The technology of cultivation, separation and purification is discussed; attempts to produce amino-acids from pure hydrocarbons are also mentioned. (50 references.)
M. SULZBACHER.

Microbial synthesis of protein from hydrocarbons. K. P. Goswami and A. Lahiri (*Chem. Age India*, 1967, **18**, 345–350).—Some 100 micro-organisms are known which are capable of assimilating one or more of 68 hydrocarbon compounds; these, for the most part, are long straight chained aliphatics capable of producing amino-acids or in some instances proteins. The development of this work is briefly described. (78 references.) K. GRAUPNER.

Relationship between vitamin E and certain sulphydryl enzymes. N. Sandler (*Diss. Abstr.*, B, 1967, **28**, 79–80).—The hypothesis that vitamin E acts by protecting essential sulphydryl groups of enzymes has been investigated. Heart, masseter, and gastrocnemius muscles of guinea pigs were used, and myofibrillar and supernatant fluid adenosine triphosphatase (ATPase) activities and supernatant fluid relaxing activity were assayed. The animals were divided into three diet groups, the normal diet being Purina guinea pig chow plus cod liver oil and olive oil. The E^+ and E^- diets consisted of a synthetic vitamin E-deficient mixture supplemented with cod liver oil. The animals in the E^+ group received in addition α-tocopherol, while the E^- animals received only olive oil. All were given vitamin C. The results support the theory that vitamin E acts to protect some essential cellular sulphydryl groups, and three possible alternative mechanisms are propounded. F. C. SUTTON.

Stabilising vitamin B_{12} analogue. Takeda Chemical Industries Ltd. (B.P. 1,080,626, 8.10.64. Jap., 14.10.63).—A vitamin solution containing a vitamin B_{12} analogue and a vitamin B_1-active compound and optionally, nicotinic acid amide and/or pyridoxine, is stabilised by the addition of a 0·2–1% by wt. of maleic acid or its water sol. Na, K, Ca, Mg, NH_4 or triethanolamine salts.
S. D. HUGGINS.

Unclassified

Review of analytical applications. *Analyt. Chem.*, 1967. **Food.** E. Barker, K. G. Sloman and A. K. Foltz (75R–92R) (746 references.)
R.J.M.

Gas chromatographic investigation of the kinetics of hydrocarbon consumption by yeasts. S. G. Kharat'yan, S. V. Vitt, Yu. M. Romanovskiĭ and V. M. Belikov (*Dokl. Akad. Nauk. SSSR*, 1967, **175**, 372–374).—Preliminary cultivation was at 30° by re-seeding (5 times) the initial culture on media containing substrates of glucose or hexadecane. Standard mixtures of hydrocarbons were used: C_{11} 14·3%; C_{12} 14·3%; C_{13} 14·2%; C_{14} 14·3%; C_{15} 14·3%; C_{16} 14·2%; C_{17} 14·2%, isoparaffin 0·5%. Strains with preliminary cultivation on glucose consumed hydrocarbon mixtures more quickly than those previously cultivated on hexadecane. Rate of consumption of the glucose group was determined by length of hydrocarbon chain; relative rate of consumption was much more varied with this group. With hexadecane group, rate of consumption at start of experiments was much slower than for the glucose group. The more volatile hydrocarbons were consumed at a greater rate than were the heavier ones. A.L.B.

Effects of a high magnetic field at different osmotic pressures and temperatures on multiplication of *Saccharomyces cerevisiae*. F. E. van Nostran, R. J. Reynolds and H. G. Hedrick (*Appl. Microbiol.*, 1967, **15**, 561–563).—Multiplication of these yeast cells when exposed to a magnetic field (*MF*) of 4,600 gauss was studied at 24-, 48- and 72-h intervals. Effect of time, temp., and osmotic pressure (*OP*) were also considered statistically. The main effect of the high *MF* was the quite marked reduction in all multiplication during each time interval. Significant interactions occurred between temp. and magnetic field at 24 h and between temp. and *OP* at each sampling interval. Synergistic effects of the *MF* and *OP* at both 28° and 38° were not significant. C.V.

Possible reactions for aflatoxin detoxification. W. Trager and L. Stoloff (*J. agric. Fd Chem.*, 1967, **15**, 679–681).—Evidence of detoxification of the four aflatoxins was obtained by means of chick embryo and tissue culture bioassays; detoxification after treatment with Cl_2, ClO_2, NO_2 and 5% NaOCl was confirmed.
P. S. ARUP.

Enzymes: a survey of modern ideas and applications [including use in food]. S. Oldham (*Mfg Chem. Aerosol News*, 1967, **38**, No. 8, 47–54, 56).—A review with 27 references. P.P.R.

Effect of irradiation on peroxidase. H. A. Al-Jasim (*Diss. Abstr.*, B, 1967, **28**, 226).—The effect of irradiation and heat on the activity of purified horseradish peroxidase and that of turnip macerate was studied; γ-rays and 1 Mev electrons were used. The concn. of the enzyme (**I**) in the reaction mixture; the pH; the effect of addition of $NaHSO_3$, glucose, $FeCl_3$, and 3-indolylacetic acid (*IAA*), were studied in relation to the radiation sensitivity of **I**, which was found to be pH dependent. $NaHSO_3$ and *IAA* both sensitised peroxidase, while $FeCl_3$ and glucose decreased its radiosensitivity. In absence of O_2 purified horseradish peroxidase was more resistant to γ-rays. F. C. SUTTON.

Turbidimetric assay for pectinase activity. G. Avigad and Y. Milner. *Israel J. Chem.*, 1967, **5**, 175–180).—Polygalacturonic and poly-methylgalacturonic acid hydrolysases (pectinases) (**I**) are determined by incubating with pectin and determining the residual pectin by complexing with cetyl-pyridinium bromide (**II**) or cetyl-trimethylammonium bromide (**III**) and measuring the turbidity. The reaction system contains final concn of 0·5% pectic acid or 1% pectin in 0·05 M acetate buffer of pH 4·5, 5mM-EDTA and a suitable dilution of the enzyme **I** in 5mM acetate buffer of pH 4·5. Incubation is at 30°; after reaction with **I** or **II**, the extinction at 400 nm is read within 1 min. The method was compared with (*i*) determination of reducing galacturonic acid end groups with Cu acetate; (*ii*) iodometric titration of reducing end groups and (*iii*) a viscometric method. The turbidimetric method and (*iii*) exhibit similar reaction patterns.

In combination with (*i*) and (*ii*), the turbidimetric method can provide information as to the type of hydrolase activity present. (21 references.) J. I. M. JONES.

Microbiological process. Glaxo Laboratories Ltd. (Inventor: E. J. Napier) (B.P. 1,048,887, 26.5.64).—An enzyme complex containing *inter alia* laminarinase and chitinase and which is suitable for a wide variety of purposes (baking, meat tenderising, chill-proofing in brewing, production of animal feeds and protein hydrolysates, bating and depilation of hides, stain removal in dry-cleaning, desizing of textiles, wound debridement, relief of inflammation, blood clots and bruises, ability to lyse pathogenic fungi, etc.) is produced by culturing *Cytophaga* NBIB 9497 on or in aq. nutrient medium under aerobic conditions. R.J.M.

Pectolytic enzymes. Belgorodsky Zavod Limonnoy Kisloty and Belgorodsky Konservny Kombinat (Inventors: V. F. Fedoseev, S. M. Dmitriev and M. A. Mehedova) (B.P. 1,081,094, 1.5.64).—A solution of cane or beet molasses which has been fermented (with *Aspergillus niger*) to give citric acid and then treated with $K_4Fe(CN)_6$ to precipitate any Fe that may be present, is treated with a water-miscible alcohol, e.g., ethanol or isopropanol, to precipitate the pectolytic enzymes; these are separated by filtration and dried at < 35°. The enzymes are used in the canning, alcoholic beverage and soft drink industries. J. M. JACOBS.

Foodstuffs. Dr. A. Wander, S. A. (Inventors: H.-J. Dietzsch, A. Krieger and A. Ciotto (B.P. 1,081,627, 23.6.65. Switz., 8.7.64).—A balanced, appetising food consists of edible artificial hollow fibres of protein, polysaccharide, soya, etc. which are of themselves digestible to a greater or lesser degree. These are filled with material such as fats, oils, sugars, fruits etc., the mixture being in liquid or paste form and the whole being digestible. The fibres are bonded together and the interfibre space is partly, or completely filled with edible material such as egg albumen, starch, gelatin, etc. J. A. SUGDEN.

Preparation of alkali *d*, *l*-malate monohydrates. Allied Chemical Corp. (B.P. 1,082,915, 31.1.66. U.S., 29.1.65).—These monohydrates $M_2C_4H_4O_5.H_2O$, in which M is an alkali metal (Na or K), are prepared by evaporating an aq. solution of the corresponding malate until its temp. is 110–130°, cooling the solution to < 30°, seeding it with crystals of the monohydrate and recovering the colourless cryst. product which remains free flowing even on exposure to a humid atm. The product can be used for the same purposes as those of other edible acid (salts). J. M. JACOBS.

3.—SANITATION, WATER, etc.

Effect of aeration on gamma irradiation of housefly pupae. B. J. Smittle (*J. econ. Ent.*, 1967, **60**, 1594–1596).—Irradiation of pupae reduced eclosion from 95% to 85% at 6,000R. Aeration with O_2, CO_2 or air caused only 2–5% deviation. Sterility of irradiated males and females was not increased by aeration with air or O_2 but CO_2 decreased it. C. M. HARDWICK.

Factors affecting the toxicity of diazinon to *Musca domestica*, L. M. Gwiazda and K. A. Lord (*Ann. appl. Biol.*, 1967, **59**, 221–232).—Diazinon [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidyl) phosphorothioate] penetrated more slowly through the integument of resistant than through that of susceptible houseflies. Traces of diazoxon [*O,O*-diethyl *O*-(2-isopropyl-4-methyl-6-pyrimidyl) phosphate] were formed when diazinon was incubated with tissue extracts of either susceptible or resistant strains. Tissue extracts of resistant, but not of susceptible flies, decomposed significant amounts of diazinon in 1 h. A. H. CORNFIELD.

Metabolism of Hempa uniformly labelled with ^{14}C in male houseflies. S. C. Chang, P. H. Terry, C. W. Woods and A. B. Borkovec (*J. econ. Ent.*, 1967, **60**, 1623–1631).—^{14}C-Hempa was injected and its metabolism analysed. 90% of injected dose was recovered from flies and excreta after 24 h ; 7 h after treatment, the flies were sterile. The identification of Hempa metabolites by thin layer, gas and radio chromatography and i.r. spectra is described and illustrated. The only major metabolite was pentamethylphosphoric triamide. (17 references.) C. M. HARDWICK.

Dieldrin in housefly central nervous system in relation to dieldrin resistance. C. H. Schaefer and Y. P. Sun (*J. econ. Ent.*, 1967, **60**, 1580–1583).—No difference was found between the rate of penetration of dieldrin into the central nervous systems of susceptible and resistant strains *in vivo* or in saline. Dieldrin does not appear to be tightly bound to tissues. After 24 or 48 h there was no indication of metabolism. The survival of resistant flies containing high levels of dieldrin suggested decreased sensitivity at the receptor site. (13 references.) C. M. HARDWICK.

Photoperiodic effect on the daily susceptibility of the housefly to trichlorfon. A. T. Fernandez and N. M. Randolph (*J. econ. Ent.*, 1967, **60**, 1633–1636).—Flies were reared in light : dark conditions varying from 10 : 14 to 24 : 0 h. Their susceptibility to residues of trichlorfon was greatest at dawn or 'lights-on' and least at dusk or 'lights-off'. (12 references.) C. M. HARDWICK.

WARF anti-resistant: DDT selection and housefly cross-resistance: A. J. Forgash (*J. econ. Ent.*, 1967, **60**, 1750–1751).—Selection with DDT + WARF anti-resistant resulted in increased resistance to DDT, diazinon, malathion, ronnel, dimethoate, Isolan, Dimetilan and pyrethins. Resistance to lindane did not increase. C. M. HARDWICK.

Joint action of SKF 525-A and sesamex with insecticides in susceptible and resistant houseflies. A. J. Forgash (*J. econ. Ent.*, 1967, **60**, 1596–1600).—SKF 525-A [2-(diethylamino)ethyl 2,2-diphenylvalerate hydrochloride] and sesamex, enhanced the action of 12 insecticides in both susceptible and resistant strains. Three other insecticides were synergised in susceptible flies only. In general SKF 525-A was more effective than sesamex as a synergist or antagonist to the insecticides. The mechanism of resistance in relation to these compounds is discussed. C. M. HARDWICK.

Chemicals tested as space repellents against yellow-fever mosquitoes. I. Esters. II. Carbanilates, benzamides, aliphatic amides and imides. T. P. McGovern, M. Beroza and H. Gouck (*J. econ. Ent.*, 1967, **60**, 1587–1590, 1591–1594).—I. Numerous esters were applied to cotton netting. The no. of days for which < 10% of mosquitoes would pass through to a human arm was recorded. Five were effective for > 100 days [dipentyl, di-isopentyl and bis(1-methylbutyl) malate, hexyl *p*-isopropylmandelate and bis(2-ethylhexyl) fumarate]. Esters with a hydroxyl group were more effective than those without one.

II. Four carbanilates, two benzamides and one acetamide gave > 100 days protection. Dipropyl 2-[(*p*-methoxybenzyl)oxy] acetamide gave 266 days protection. C. M. HARDWICK.

Resistance in *Aedes aegypti* to chemosterilants: effect of Apholate selection on resistance to Apholate, Tepa and Metepa. R. S. Patterson, C. S. Lofgren and M. D. Boston (*J. econ. Ent.*, 1967, **60**, 1673–1675).—Selection over 43 generations from *A. aegyptii* larvae exposed to 5-25 ppm Apholate, produced 20-fold resistance. Larval mortality was low during a 5-day treatment but at higher doses development was retarded and pupal and adult mortality was higher. There was no difference between the sexes. Cross resistance to Tepa and Metepa was minimal but suggested incipient resistance. C. M. HARDWICK.

Teratogenic effects of a tranquiliser, trifluoperazine dihydrochloride on yellow-fever mosquito. M. S. Quraishi (*J. econ. Ent.*, 1967, **60**, 1650–1655).—Larvae of *Aedes aegypti* were treated with solutions of the tranquiliser (2–75 ppm). The resulting abnormalities to the mouthparts and head sclerites of the adults are described. At 75 ppm eclosion was also affected. (13 references.) C. M. HARDWICK.

Effect of certain factors on vapour phase sterilisation of *Bacillus subtilis* ATCC 9524. Tien-szu Liu (*Diss. Abstr.*, B, 1966, **27**, 1505).—Spores of *B. subtilis*, carried on paper discs, were exposed to pure ethylene oxide (**I**) at various temp. Pressure and R.H. are shown. Spores of this organism, on paper and glass discs, were also exposed to a gaseous mixture (12% **I** and 88% CF_2Cl_2) at various temp. The effect of pH on resistance to **I** was studied. Citric acid and phosphate buffer solutions (as spore suspending media) were examined at varying pH values. The concn. of buffer influenced the resistance of the spores. F. C. SUTTON.

Disinfectants in the beverage industries. L. Vanossi (*Industrie Aliment., Pinerolo*, 1967, **6**, 78–80).—The merits and disadvantages of different bactericides and bacteriostats are summarised. Hypochlorites, chlorine and Cl-deriv., phenols, iodoform, surface-active agents, and heavy metal salts are considered. C. A. FINCH.

Pitfalls of disinfectant testing. A. B. Law (*Soap chem. Spec.*, 1967, **43**, No. 9; 84, 86, 90, 94, 129–130, 132).—The difficulties encountered in the laboratory determination of germicidal effectiveness are examined. Examples are given showing the misleading results obtained in standard tests. G. R. WHALLEY.

Microbial destruction by low concentrations of hypochorite and iodophor germicides in alkaline and acidified water. H. Hays, P. R. Elliker and W. E. Sandine (*Appl. Microbiol.*, 1967, **15,** 575–581).—Test organisms included *Escherichia coli, Streptococcus lactis, Lactobacillus plantarum, Pediococcus cerevisiae,* and *Saccharomyces cerevisiae.* Except where the hypochlorite contained excess alkalinity both groups of germicide showed similar rates of destruction at equivalent concn. for the bacterial species tested but the hypochlorite (I) responded more readily to a downward shift to pH 5·0 than did the iodophors (II) although these compounds gave consistently greater destruction of yeast cells than I. Succession treatments with low levels of II (6 ppm), followed by I (12–25 ppm) resulted in high level destruction of all test organisms, suggesting that this technique might be advantageous. *S. lactis* was specially resistant and proved a useful test organism for the comparative effectiveness of these halogen preparations. C.V.

Small chamber tests of ethylene dibromide and ethylene dichloride on adult grain-infesting coleoptera. C. R. Ellis and F. P. Morrison (*Can. J. Zool.*, 1967, **45,** 435–448).—A cheap small-chamber fumigation apparatus and a technique resulting in reduced day-to-day variation in results are described. The LD_{50} values of a strain fumigated on different days varied as much as 40%, whereas the ratios of a standard and test strain fumigated on the same day varied less than 6% on different days. The natural mortality of *Sitophilus granarius* fed 10% sugar solution was less than that of those fed wheat grains. Sugar solution fed before exposure made the weevils more susceptible but when it was fed during the post-exposure period it increased the observed LD_{50} by 60%. E. G. Brickell.

Quinoid secretions in grain and flour beetles. R. K. Ladisch, S. K. Ladisch and P. M. Howe (*Nature, Lond.*, 1967, **215,** 939–940).—Reports and discusses polarographic detection and determination of quinone (I) and quinol (II) in 17 species of Tenebrionid beetles which live on stored food. Normal concn. of I range up to ~ 150 μg per beetle, but max. concn. of 384 μg (~ 5% of body wt.) were found in *Tribolium brevicornis* and *T. madens.* In some beetles the secretion contains only II; most secretions are simple alkyl *p*-benzoquinones similar to those found in *T. castaneum,* but there are large quant. differences among species and in individuals of any one species. Based on a survey of 95,390 tons of stored sunflower seeds contaminated with 18×10^9 *T. castaneum* (*J. Stored Prod. Res.*, 1965, **2,** 69), it is calculated that the food is in contact with 2200 lb of I and 290 lb of II. W. J. Baker.

4.—APPARATUS AND UNCLASSIFIED

Growth response of *Lactobacillus brevis* to aeration and organic catalysts. J. R. Stamer and B. O. Stoyla (*Appl. Microbiol.*, 1967, **15,** 1025–1030).—Under stationary and anaerobic conditions greater cell yields were obtained from autoclaved than from filter-sterilised glucose media. Fructose was tentatively identified as the product generated during the heating and addition of micromol. quantities of pentoses or pentose precursors to the filter-sterilised medium was equally effective in stimulating growth. (16 references.) C.V.

Changes in official methods of analysis made at the 80th annual meeting (A.O.A.C.) October 10–13, 1966. Anon. (*J. Ass. off. analyt. Chem.*, 1967, **50,** 190–242). A. A. Eldridge.

Determination of small amounts of tin in organic matter. I. Amounts of tin up to 30 μg. Analytical Methods Ctee. (*Analyst, Lond.*, 1967, **92,** 320–323).—The recommended method consists first in destruction of org. matter by wet oxidation with acids, adjustment of the resulting solution to be 9 N in H_2SO_4, followed by selective separation of Sn as SnI_4 and its extraction into toluene. The Sn^{4+} is determined spectrophotometrically at 552 nm by the catechol violet procedure in solution buffered to pH 3·8 with acetate. W. J. Baker.

Determination of small amounts of zinc in organic matter. Analytical Methods Ctee. (*Analyst, Lond.*, 1967, **92,** 324–325).—The two procedures recommended are (after destruction of org. matter): (*a*) the titrimetric dithizone method for ≯ 100 μg Zn and (*b*) the spectrophotometric dithizone method for ≯ 10 μg Zn in the final solution. These procedures are those previously recommended by the Committee in '*Determination of trace elements, with special reference to fertilisers and feeding stuffs*', (W. Heffer & Sons, Ltd., 1963, pp. 34–36), but slight operational modifications are proposed. These, with reasons for their adoption, are stated. W. J. Baker.

Solvent extraction and determination of magnesium in biological materials. K. J. Hahn, D. J. Tuma and M. A. Quaife (*Analyt. Chem.*, 1967, **39,** 1169–1171).—After digestion of the sample with HNO_3, acetate buffer and Na diethyldithiocarbamate are added to the solution, which is then extracted with $CHCl_3$ to remove heavy metals as dithiocarbamates. The aq. phase is adjusted to pH ~ 9 and extracted with thenoyltrifluoroacetone (I) in C_6H_6–tetrahydrofuran to remove most of the Na. The Mg complex in the org. phase is re-extracted into N-HNO_3 and irradiated to form ^{27}Mg, the 0·84 MeV peak being used to calculate the Mg concn. The activity count is made after removal of residual traces of ^{24}Na by a further extraction with I. There is no interference from Mn, Ca (in serum) or Al (which also forms ^{27}Mg on irradiation). For up to 60 μg of Mg, the coeff. of variation is 3·4%. (12 references.) W. J. Baker.

Spectrographic determination of brucine. R. Dörr (*Z. analyt. Chem.*, 1967, **226,** 417–424).—The efficiency of various processes for isolating brucine (I) is established, and methods are devised for its determination in plant material and nutrient solutions. The final spectrophotometric measurement is made on a 0·1 N HCl solution at 262 nm; 0·1–2·5 mg of I per 100 ml of final solution can be determined. A. Townsend.

Agricultural applications of some polypropylene products. F. Barbieri Hermitte (*Materie plast.*, 1967, **33,** 489–494).—Applications of heavy denier polypropylene (monofilament and multifilament) in ropes, hessian, nets and similar uses are compared. Methods of replacement of sisal and manila are described. The properties of monofilaments of polypropylene, polyethylene, polyesters, and nylons are compared with those of manila and sisal. Relative economics are discussed briefly. C. A. Finch.

Assessment of chemical resistance of enamels [in cookers etc.]. H. Ankerst (*Glas.-Email-Keramo-Tech.*, 1967, **18,** 119–127).—Service performance cannot be assessed by any one test. Design (horizontal or vertical) plays an important part while accumulation of corrosion products will hasten the process. Certain products are specially mentioned: pork and onion, brown beans, red cabbage and potato with salt can exert a specific corrosion effect. A method for testing TiO_2-containing cooking enamels, which includes three laboratory, direct chemical erosion tests, is discussed. It is noted that the addition of colour to an otherwise satisfactory frit may result in an adverse effect and that Co-aluminate blue is found to give the best results. J. A. Sudgen.

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ABSTRACTS

JULY, 1968

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

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